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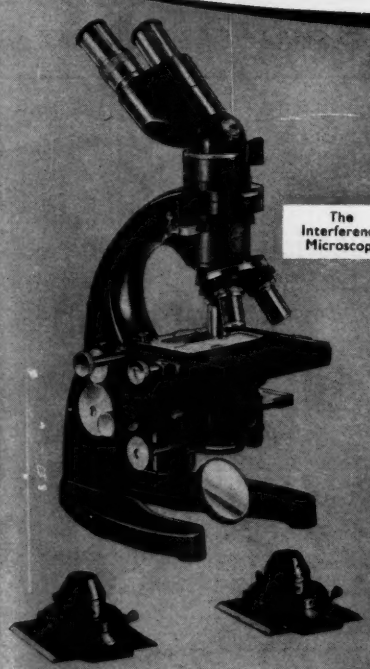

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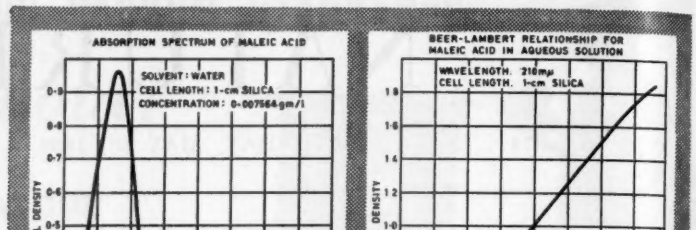

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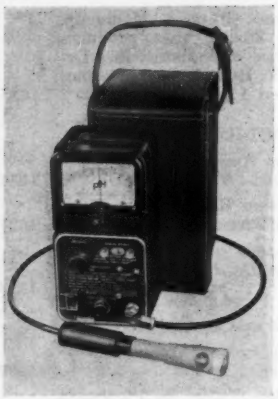

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RECRUITMENT IN GOVERNMENT SERVICES AND SECURITY REGULATIONS

THE White Paper dealing with defence is this year entitled "Progress of the Five-Year Defence Plan"* and is much shorter than last year's White Paper. "Defence Statistics 1959/60"†, however, have been collected in a separate White Paper, and there was also issued, in July 1958, a further White Paper, "Central Organisation for Defence"‡. The Defence Budget is given as £1,502·13 million net, of which £585·46 million is on research and development. There is no separate entry under this heading for the Ministry of Defence, but for the Admiralty, the War Office, the Air Ministry and the Ministry of Supply the respective net figures for research and development are £169·03 million, £53·68 million, £213·85 million and £148·90 million. Gross expenditure under this heading by the Ministry of Supply is £521·60 million.

Despite these figures there is, however, no other specific reference to research in any of these White Papers. There is, in the one reporting on progress, a reference to the valuable exchange of information with the United States which has permitted important technical advances to be made in the design of nuclear war-heads, and it is stated that after a recent review of the British ballistic rocket programme, in the light of technical developments in Britain and in the United States, the Government reached the conclusion that, on present knowledge, *Blue Streak* is the type of missile best suited to British needs. It is obvious that the modernization of the Fleet and the re-equipment of the Army are both based on much technological research, but the magnitude of this effort is nowhere disclosed, nor is there any indication as to how effectively it is co-ordinated.

On the Defence Research Policy Committee there is again silence, nor is the Committee even mentioned in the White Paper dealing with the central organization for defence. The latter paper formally announces the confirmation, as a result of eighteen months experience, of the re-organization of the Ministry of Defence announced by the Prime Minister on January 24, 1957, with the introduction of three new features. First, the composition and operation of the Defence Committee of the Cabinet are being adjusted to secure greater flexibility and efficiency. Secondly, a Defence Board is being set up to assist the Minister of Defence in formulating a defence policy and in dealing with inter-Service problems. Some adjustments are also being made in the staff organization, including the creation of a new post, Chief of the Defence Staff, in place of the existing combined posts of Chairman of the Chiefs of Staff Committee and Chief of Staff to the Minister of Defence. The holder of this post will be responsible to the Minister of Defence and will be his principal military adviser. He will also be a member of the Defence Board, of which the Minister of Defence will be chairman. The other members of the Board are the Ministers for the three Service Departments, the Minister of Supply, the Chief of the Naval Staff, the Chief of the Imperial General Staff, the Chief of the Air Staff, the Permanent Secretary of the Ministry of Defence and the Chief Scientist of the Ministry of Defence. As already noted, there is no reference to the Defence Research Policy Committee, nor, although in the defence statistics the interrelation of defence and civil expenditure is fully recognized, to the Advisory Council on Scientific Policy. Formal or even informal arrangements for the correlation of defence and civil requirements in the research and development effort in Britain appear to be lacking.

This aspect of the defence effort was scarcely discussed in the subsequent Parliamentary debates although the dependence of effective defence on scientific and technical advance was generally recognized. There was, however, a significant admission by the Minister of Supply, Mr. Aubrey Jones, in replying for the Government in the debate in the House of Commons on February 26. While the Minister of Defence had given most encouraging accounts of recruitment to the Armed Forces, Mr. Jones admitted he could not be quite so encouraging about the recruitment of scientists to the Government's defence establishments. He could offer no improvements in pay or amenities, but he hoped that due advantage would be taken of the unrivalled opportunities for research which he could offer.

Even more significant, however, was Vice-Admiral J. H. Hallett's reference to secrecy. Vice-Admiral Hallett was concerned primarily with the modernization of the Fleet and whether this was being carried out appropriately for the functions it would perform, particularly in respect of the defence of communications and the threat of submarine warfare. He pointed out, however, that no one outside a small circle of Service officers, Government officials and possibly senior Ministers could hope to answer the vital questions because they did not possess the information which is the essential basis of any opinion worth while.

That is equally true of any other section of defence policy, and Admiral Hallett reminded the House of Commons that this is a novel and dangerous position. In the past, military thinking in Britain has been enriched by the contributions of people outside the Services, outside the Government and outside politics and public life altogether. Often such con-

* Progress of the Five-Year Defence Plan. Pp. iii+7. (Cmnd. 662.) (London: H.M. Stationery Office, 1959.) 9*d*. net.

† Defence Statistics 1959/60. Pp. 8. (Cmnd. 661.) (London: H.M. Stationery Office, 1959.) 9*d*. net.

‡ Central Organisation for Defence. Pp. 6. (Cmnd. 476.) (London: H.M. Stationery Office, 1958.) 6*d*. net.

tributions have profoundly influenced the thought of junior officers, who incidentally themselves had access to the information on which to reach independent judgments of their own.

That, said Admiral Hallett, has all changed, and although such writers are still to be found, their contributions are now based essentially on guesswork, and very few people indeed possess the vital information without which the study of tactics and strategy is waste of time. The great danger, moreover, is that the handful of experts who alone possess the information are not necessarily the people best qualified to interpret it. Admiral Hallett illustrated this by our experience in the development of radar, when the secrecy with which this was developed led to its applications in naval warfare being overlooked, and he urged the Government to consider very seriously whether the present secrecy is any longer in the true interests of national security.

The impossibility of any effective discussion of the scientific and technical aspects of defence policy in the light of information publicly available was pointed out in these columns in commenting on the White Paper on defence last year. It is obvious that Admiral Hallett is uneasy as to the situation from the Services point of view and his disquiet is based, moreover, on his experience as vice-controller of the Navy and as second-in-command of the Anglo-American Striking Fleet. Indeed, experience in the latter post made him particularly anxious as to the effect of secrecy on the Anglo-American exchange of scientific and technical experience.

These comments seem to reinforce doubts as to whether the Defence Research Policy Committee is functioning effectively, and as to its relations with the Advisory Council on Scientific Policy. Even with adequate informal contacts, the present structure appears to place an excessive responsibility on the common chairmanship, and makes informal discussion in scientific and technical circles not merely desirable but even essential. This is as important from the point of view of efficient use of the limited resources of Great Britain both of materials and of man-power as it is from that of the wise direction of policy, and would go far to establish the public confidence which is always an essential element in effective defence. To say the least, Mr. Orr-Ewing's reply to a question to the Minister of Defence on April 8 as to what consideration the Defence Research Policy Committee has given to the effect of the resignation of scientific research workers as a result of the uncertainty of Government policy is not helpful. Conformity with established practice is no longer a sufficient reason for secrecy and a refusal to promote public discussion. Secrecy may be one reason why the Minister of Supply finds it difficult to recruit sufficient scientists for his research establishments. Even unrivalled material facilities and equipment will not attract first-class minds if they are doubtful as to the value of their work, or the possibility of the free discussion of results among their fellows with the cross-fertilization of minds upon which scientific advance depends.

PRESENT TRENDS IN BOTANICAL STUDIES

Vistas in Botany

A Volume in Honour of the Bicentenary of the Royal Botanic Gardens, Kew. Edited by Dr. W. B. Turrill. (International Series of Monographs on Pure and Applied Biology. Division: Botany, Vol. 2.) Pp. xv+547. (London and New York: Pergamon Press, 1959.)

An occasion such as the bicentenary of the Royal Botanic Gardens, Kew, offers a rare opportunity to take stock of the present position in botany and of desirable trends for the future [see also p. 1488 of this issue]. The present volume, edited by W. B. Turrill, represents an attempt to do this. It consists of surveys of different facets of botanical inquiry by well-known authorities, and though perhaps it emphasizes those aspects of the subject which have especially been the pre-occupation of Kew, it covers so wide a field that there are few botanists who will not feel constrained to consult its pages and few who will put it down without having profited by its perusal.

Without any wish to make invidious comparisons, which would indeed require unprecedented knowledge in a variety of subjects, it is evident that the distinguished authors of these pages have looked at their limited fields in very diverse ways. Some have succeeded in discovering and presenting a fair and attractive vista; others perhaps have scarcely been able to see the wood as a whole but have not missed a tree, while still others have been so interested in the first tree that they have scarcely gone beyond it. In each of the several styles there is at least one example which is masterly of its kind, and the diversity of treatment adds piquancy to one's reading and is a welcome change from the monotony of a series of 'reviews of the present position'.

The plan of the work thus is a series of surveys of the main groups of plants coupled with special statements about the more general botanical problems. There is a historical preface by the Editor, and the first paper is a short introductory one by the present director of the Royal Botanic Gardens, Kew, Dr. George Taylor.

The massive contributions of Kew to taxonomy justify pride of place being given to an article on this subject by H. J. Lam. This ranges widely over what may perhaps be called the basic theories underlying classification, taxonomy and systematics, with a sufficiently individual point of view, perhaps fundamentally classical and morphological, to integrate wide reading with interesting and suggestive reflexion. Rudolfo E. P. Pichi-Sermolli also accepts taxonomy as the synthesis of all biological knowledge and exemplifies this point of view in his exhaustive treatment of the Pteridophyta, in which, after surveying the development of present knowledge, he delves deeply into the problems of phylogeny in the various taxa of this great group. In dealing with the Gymnosperms, W. B. Turrill, apart from a discussion of the 'telome' hypothesis, has to accept the limitations due to modern conceptions of the Gymnosperms as a group of phyla offering vistas of several diverse types, in which the peculiarities of the reproductive systems in comparison with the Angiosperms occupy a prominent place. P. W. Richards records much of interest in the Bryophyta, especially to ecologists and

morphogeneticists, in relation to the developmental stages, the alternation of forms and their evolution. The Algae are considered by W. R. Taylor, "Phycology in Retrospect and Anticipation", who directs attention to the enormous gaps existing even in the taxonomy of the group from which the higher plants must have evolved. Particularly can one note the lack of an organized embryology and even of essential cytological detail. In contrast there is a high degree of knowledge in some limited fields of algal physiology, ecology and utilization. C. T. Ingold's admirable survey of the very extensive literature on Fungi shows how much studies of this group have contributed to general botanical problems, particularly perhaps those of sex and inheritance, while the physiology of their nutrition has much to give to problems of morphogenesis through hormone control, as well as to knowledge of antibiotics. K. A. Bissett has faced with success the difficult task of treating the bacteria as organisms rather than as biochemical or medical entities.

While the study of the larger groups of plants and their taxonomy has been pursued for many years, that of the viruses, if indeed they are to be classified as plants, is comparatively recent, and as F. C. Bawden points out, has only made much progress in the past two or three decades with the rapid advances in the chemical and physical methods developed for enzymology. The fascinating story of their crystal structure and intimate relation to other nucleoproteins has made the viruses of even greater interest to the general biologist than to the plant pathologists who first recognized them. This is equally true of the details of cytological structure revealed by D. G. Catcheside, who, switching skilfully from cytoplasm to chromosomes and bacteriophage, marshals the evidence that deoxyribonucleic acid is the significant part of the nucleus providing genetic information. These two papers are, in fact, a valuable source of ideas for those not very familiar with rapidly advancing fields of work. Also essentially analytical in outlook is the article on biochemistry by E. C. Bate-Smith. Perhaps the most interesting part of this is an appreciation of the possibilities of comparative biochemistry in plants which, it is thought, will be based on secondary products rather than those common to all organisms. The useful biochemical processes for the purpose of phylogenetic comparisons would thus be those present in a series of forms, but lost and not regained in their successors, as apparently may be the case in some of the leucoanthocyanins.

A long period of inertia followed the early anatomical discoveries of Grew and Malpighi, and in tracing the re-development of plant anatomy, C. R. Metcalfe illustrates the leading part played by the Jodrell Laboratory at Kew in Great Britain. Perhaps more important than his plea for "brighter" anatomy teaching is the need he stresses for organizing the enormous amount of material which must be available for comparative use, particularly perhaps in the Angiosperms. Another subject, palæobotany, profited greatly from the activities of the Jodrell Laboratory under D. H. Scott, and indeed from the fact that three of the directors of Kew, Sir Joseph Hooker, Sir Arthur Hill and Sir Edward Salisbury, have engaged in palæobotanical research. John Walton's sympathetic account of the personalities who have led the way in this field in Britain, and of their views, must be in many ways the best of the pure 'vistas', Sewardian in quality.

If some of the more modern aspects of the subject illustrate the value and the use of the analytical method, the considerable paper, "Genes, Chromosomes and Evolution", by G. L. Stebbins, offers in contrast an approach through the widest fields of cytogenetics and floristics which has also become popular in the past twenty years and which aims at integrating the facts of distribution, taxonomy and cytology in terms of the most recent genetical theory. Plant geography, as W. B. Turrill shows in a notable and comprehensive treatment, also integrates a great deal of material of general and evolutionary interest, as well as seeking to classify distributional units and arrange them in provisional and more useful hierarchies. The characteristics of plant sociological units and their groupings are considered in greater detail by J. Braun-Blanquet ("Grundfagen und Aufgaben der Pflanzensociologie") together with their relations to synecology, to community succession and (somewhat sceptically) to statistics. Causal plant ecology is treated by a former director of Kew, Sir Edward Salisbury, who considers it as running parallel to physiology, the relation of plant growth to physical and chemical environmental characters, but subject to the additional stresses imposed by competition. In these circumstances, extremes of environment, particularly in relation to phases of growth, are often more decisive in nature than average conditions, while rooting conditions are especially important in competition. Appropriately in this connexion, the paper on plant physiology by F. A. C. Went lays emphasis on the control of plant growth by temperature regimes, by photoperiodism, and by hormones, as well as directing attention to the tendency to locate individual chemical processes in special localities, either in the cell or in special sites in the tissues.

A comprehensive volume of this type will always leave the reader with at least some impressions about the subject surveyed. Biologists can rarely get far away from the central topics of evolution and taxonomy, nowadays perhaps accompanied by the study of the mechanisms of heredity, and these ensure a considerable integration of any large series of general botanical papers such as this. But the reader is also made aware of what is becoming an increasingly important aspect of biological work, the physical difficulty, to say nothing of the intellectual one, of maintaining an organization in which the vast accessions of material and knowledge can be stored in a readily available form and integrated for general use. It seems without question that this is only possible in an institution such as that at Kew, and one is able to appreciate properly how an organization based on gardens, herbaria and laboratory has done so much for taxonomy in particular and for botany in general. Whether this is the organization most suitable for the widest type of integration is no doubt a question which would require very detailed discussion. Perhaps the second point emerging from this survey of the botanical field is the very large gap remaining in our knowledge of the relations between growth and morphological development, and particularly of the chemistry and mechanics of developing cells and tissues. The nature of the effects of genes on details of morphology is likely to remain very largely a matter for speculation alone, until some at least of these gaps are filled. Here perhaps we may leave a volume which contains many important contributions to botanical thought.

W. H. PEARSALL

HARVEY ON MOVEMENT IN ANIMALS

De Motu Locali Animalium, 1627

By William Harvey. Edited, translated and introduced by Dr. Gweneth Whitteridge. Pp. xii+163. (Cambridge: At the University Press, 1959. Published for the Royal College of Physicians.) 60*s*. net.

SIR RUSSELL BRAIN was president of the Royal College of Physicians when plans were being evolved to mark the Tercentenary of Harvey's death. *Inter alia*, proposals were made to the College Library Committee at one or more of its meetings that Dr. Gweneth Whitteridge, who is archivist to Harvey's Hospital of St. Bartholomew in the City of London, should first transcribe and later translate and duly introduce his "De motu locali animalium", which forms *ff*. 69–118 of the British Museum manuscript Sloane 486. This was written throughout in Harvey's own hand, and consists of his notes on the anatomy of muscles (*ff*. 1–68), followed by his "local movement in animals". A title, "Guilielmus Harveius de musculis, motu locali etc.", written on *f*. 1 of the double manuscript, may have been added by Sir Hans Sloane. Anyhow, Harvey's rough draft for his intended book on the local movement of animals was begun in 1627, was mentioned in *Cap. xvii* of his "Exercitatio anatomica de motu cordis et sanguinis in animalibus", 1628, and was added to on several occasions, but was never cast into final literary form. The notes, which came to light in the British Museum in the middle of the nineteenth century, have never before been transcribed, but they make clear the tenor of Harvey's projected work on muscle and, though containing no new experimental observations, show that his understanding of muscle and muscular contraction was sounder than that of his predecessors and even of some of his successors.

The work has been published for the Royal College of Physicians at the Cambridge University Press, and has as an illustration a photograph of *f*. 95 of the manuscript under ultra-violet light, as evidence that such treatment has not revealed the presence of any writing which was not previously visible. The edition is limited to 1,000 copies.

Its chief concern has been to provide a text which is readable. The English version of "De motu locali animalium" is not to be taken as a literal translation of the Latin text, for it was decided against following Harvey's Latin literally and reproducing his notes in note form in English. The alternative method adopted was to follow the arguments alluded to by Harvey and to expand his notes into complete sentences by reading the books which he was following, and as closely as possible completing the thoughts which he, for his own reference, needed only to note down in a few words. Inevitably, this method has led to a certain amount of interpretation which may or may not be correct. The English version is therefore offered merely as a suggested rendering.

Dr. Whitteridge thanks the Librarian and staff of the Library of the University of Edinburgh, which has on its shelves several of the actual editions of the works used by Harvey. She also thanks her husband, Prof. David Whitteridge, for his discussion of physiological problems and for his help in writing about the importance of Harvey's 1627 treatise in the history of muscle physiology. She further expresses gratitude to Dr. Lorenzo Minio-Paluello for his encouragement to one who, before this, knew nothing of the natural philosophy of Aristotle.

There can be no doubt that Dr. Whitteridge has done consummately well in transcribing Harvey's obscure handwriting, translating his Latin, and annotating his notes; as Sir Russell Brain says, she has earned the gratitude and admiration of students of Harvey all over the world, not least among them being the President and Fellows of the Royal College of Physicians of London, at whose request she undertook the work.

Though Harvey's notes, as already stated, contain no great discovery, they have "much of interest to the student of his mind. They show him, as always, gathering his illustrations from every kind of animal and even plant life, and in addition recalling clinical observations which must have impressed him".

K. J. Franklin

RECONSTRUCTING THE PAST

Life and Death in the Bronze Age

An Archæologist's Field-Work. By Sir Cyril Fox. Pp. xxvii+193+49 plates. (London: Routledge and Kegan Paul, Ltd., 1959.) 45*s*. net.

THIRTY years ago prehistorians were content to collect the relics left by early man, and to accept the view that little, if any, knowledge of the life he lived could be obtained—they were satisfied to produce the skeleton, but fearful of attempting to clothe it with flesh and blood. It is true that occasionally—as, for example, when discussing palæolithic ceremonial burials or when the cave art was under consideration—it did seem permissible to talk about ritual and magic, but by and large it was felt to be dangerous to attempt to picture prehistoric man as a living individual, even with the help of such a kindred study as ethnology. How could one be certain that similar conditions in times very different must have produced a similar outlook on life?

For some time past, however, a new approach to the study of prehistoric man has been developing, leading to just that ground where the earlier investigators feared to tread. This new approach can be dangerous and result in grave errors, but not when it is pursued by such a master of the subject as is Sir Cyril Fox. There is no doubt that if a brilliant investigator has lived with his work for much of a lifetime he develops a sixth sense which enables small circumstances to be noticed which fall severally into place until the intuition becomes a well-nigh demonstrable fact. This is what makes any work by Fox so fascinating. Any good excavator can dig up prehistoric relics in a highly satisfactory manner using all the modern techniques; but it takes a Fox so to live with the builders of, say, a megalith he is investigating as to see them at their work of building and performing the ritual for which the monument was raised—and then to convey all this to his readers. Fox has another advantage over many of his contemporaries in that he remains so young. Often the later, post-retirement, works of a savant make sad reading, but this is not at all the case with the book under review. There is a freshness about it all, even when mere descriptions of excavations are being given, which is stimulating and delightful.

The period under consideration covers the millennium 1700 B.C.–700 B.C., and the excavations in question were mostly situated in Wales and were mainly

the author's own work. The volume is splendidly illustrated with plans, line blocks and half-tones. The chapters are arranged geographically—north Wales, west Wales, west and south Wales, the 'Food-vessel' phase in Britain, barrows in Glamorgan, etc. To start with there is a long introduction to the subject. Here the problems of the Bronze Age in general and barrows in particular are discussed and distributions considered. The stage is thus set for the account of a round barrow in Ysceifiog parish, North Wales, and at once we are made to see what must have happened there in the past.

Sites in the other geographical areas are treated in the same manner and we are thus given a vivid picture of how and in what order the processes connected with barrow-building took place—facts of construction that, having been appreciated, have sometimes "formed a basis for the recovery of an elaborate ceremonial". The various finds—pottery, tools, and the like—are, of course, carefully noted and their parallels elsewhere discussed. The Welsh monuments are not treated *in vacuo*; the importance of their geographical position *vis-à-vis* Ireland is stressed. "Life and Death in the Bronze Age" is well worth careful study not only for the many facts brought to light but also, as has been already said, for the vivid picture it affords of happenings which must have taken place in a millennium of the British Bronze Age.

M. C. BURKITT

THE EARLIEST GEOLOGICAL TREATISE

The Earliest Geological Treatise (1667)

By Nicolaus Steno (Niels Stensen). Translated from "Canis Carchariae Dissectum Caput", with Introduction and Notes by Dr. Axel Garboe. Pp. v+51+4 plates. (London: Macmillan and Co., Ltd., 1958.) 15*s*. net.

MODERN geology as an inductive science was born in Italy in the latter part of the seventeenth century, the most famous of the pioneers being Nicolaus Steno (Niels Stensen, 1638–86), "the learned Dane", who had settled in Florence. In 1669 appeared his "De solido intra solidum naturaliter contento dissertationis prodromus" (the Prodromus to a dissertation concerning solids naturally contained within solids), the importance of which was recognized by Henry Oldenburg, secretary of the Royal Society, who published a translation (London, 1671).

Steno was led to study rocks through the examination of a shark's head which had been sent to him for dissection, and his account, "Canis Carchariae dissectum caput", published two years before the Prodromus, contains an appendix, which Dr. Garboe has translated, in which some of the essential elements of the later work are set down.

Recognizing the 'tongue stones' (*glossopetrae*) found in the rocks of Malta to be in all respects similar to the teeth of living sharks, he argues cogently that these and other fossils are the remains of once living aquatic animals, and he combats the view then commonly held that these bodies had grown within the rocks. This appendix consists of eleven 'observations' of sedimentary rock features, followed by six 'conjectures' in which the origins of the sediments, their fossils, cross-cutting veins, and departure from horizontality are deduced.

It seems strange to the geologist of to-day that the reaching of such fundamental and seemingly obvious conclusions was so long delayed, but their apparent conflict with the teachings of an all-powerful Church not only hindered the recognition of the significance of the evidence but also made men wary of voicing their opinions.

Dr. Axel Garboe is to be thanked for this page-by-page translation, which makes available to the English-speaking world "the earliest geological treatise". As frontispiece there is a reproduction of the portrait of Steno as a young man (Uffizi Gallery, Florence); also there is a copy of the title page of the 1667 work and its figure of a shark's head and teeth.

L. HAWKES

PRINTED CIRCUITS

The Technology of Printed Circuits

The Foil Technique in Electronic Production. By Dr. Paul Eisler. Pp. x+405. (London: Heywood and Co., Ltd., 1959.) 60*s*. net.

PRINTED circuits have been developed in the past decade for use in equipment ranging from electronic computers to aircraft de-icing systems, and continue to replace manual wiring. In conjunction with component insertion machines they enable high rates of production to be attained with low labour and inspection costs, and a greater uniformity of product than was possible by previous methods of wiring.

The book gives ample evidence of the author's position as a leading contributor to the technology of printed circuits, and the inventor of the printed foil technique now in general use.

The first two chapters describe early attempts at printed circuit production, and alternative methods such as spraying, stencilling, die-stamping and plating.

Chapters 3–8 contain a full account of the large-scale manufacture of printed circuit boards, including the photography of the master drawing, the preparation of the raw material, the printing processes, and etching and cleaning. Chapter 9 gives details of the subsequent operations such as the deposition of protective coatings, soldering and encapsulation.

Chapter 10 contains an excellent description of the small-scale production of printed circuits, and the laboratory apparatus required.

The following three chapters deal with the design of printed circuits, the selection of foil and insulating board, the choice of components, automatic assembly and fault finding.

The final chapters describe methods of reducing the weight and size of electronic equipment, the use of printed components, and the application of printed circuits to microwave systems and electronic devices. An excellent bibliography is included.

The wealth of practical detail given in the early chapters will be invaluable to those engaged in the manufacture and design of printed circuit assemblies, but the general reader would welcome more information about the use of printed circuits in various branches of the electrical industry, and their influence on system design, mechanical lay-out, and maintenance procedure.

However, this is the most comprehensive treatment of the subject generally available, and it is clearly destined to become the standard work of reference on printed circuit technology.

J. C. CLULEY

BICENTENARY OF THE ROYAL BOTANIC GARDENS, KEW

By Dr. W. B. TURRILL, O.B.E., F.R.S.
Formerly Keeper of the Herbarium and Library

THE choice of 1959 as the bicentenary of the Royal Botanic Gardens, Kew, is approximately a correct one so far as the present Gardens are concerned. There is at least one reference to a garden at Kew belonging to the Rev. William Turner (*c.* 1510–68), who has been termed 'the father of English botany'. Sir Henry Capel (?–1696) was also a notable cultivator of plants, but mainly from the horticultural rather than the botanical point of view. His property at Kew descended to a grand-niece, Lady Elizabeth Capel, who married Mr. Samuel Molyneux, a politician and secretary to the Prince of Wales (later George II). On the death of Molyneux's widow in 1730, the then Prince of Wales, Frederick Louis, obtained a lease of Kew House and laid out the grounds afresh. Frederick married the Princess Augusta of Saxe-Gotha in 1736 and died in 1751. The Princess Augusta, then Dowager Princess of Wales, continued to live at Kew, and about 1759, with the help and advice of John Stuart, third Earl of Bute (1713–92), who was sincerely interested in botany, laid out about nine acres of the grounds of Kew House as a botanic garden and appointed the architect, Sir William Chambers (1726–96), to embellish the garden and the adjacent pleasure grounds. Many of the buildings erected by Chambers have now disappeared, but several remain, of which the most noteworthy are the Orangery (1760–61) and the Pagoda (1761–62).

Perhaps the most important happening, from the practical point of view, of this period was the appointment in 1759 of William Aiton (1731–93) as superintendent of the botanic garden. Aiton was a botanist as well as a gardener, and in 1789 published his "Hortus Kewensis", or a "Catalogue of the Plants Cultivated in the Royal Botanic Garden, Kew". This work, in three volumes, includes 5,500 species which are named, often described, and classified according to the Linnean system.

Aiton, at first, had under his charge only the relatively small botanic garden of the Princess Augusta; but his responsibilities were increased more or less gradually, and in 1784 he took over the entire management of both the Royal Gardens of Kew and Richmond. William Aiton died in 1793 and was succeeded by his eldest son, William Townsend Aiton (1766–1849), who published a second edition of the "Hortus Kewensis" in five volumes.

While the Aitons were head gardeners, superintendents, or what would now be called curators of Kew, it was first to the Earl of Bute and later to Sir Joseph Banks that the development of Kew on the purely botanical side was very largely due. The former was unofficial director of Kew under the Princess Augusta. The deaths of his father and grandfather (George II) brought the Kew and adjacent Richmond properties into the ownership of George III, and in 1765 the King obtained powers from Parliament to close the public right of way, known as Love Lane, which separated the two "Gardens". Though the closure was not completed for some years, it was from this fusion of the gardens (botanic and pleasure) of Kew House and part of those of Richmond Lodge that the present title of Royal Botanic Gardens (in the plural) originated. George III, Queen Charlotte, and their family (fifteen sons and daughters) spent much time at Kew, where the King delighted to play the part of 'Farmer George'.

When the Princess Augusta died in 1772 her property came immediately and fully into the possession of George III, who replaced the Earl of Bute by Sir Joseph Banks as adviser on the botanical developments at Kew. Banks planned to grow species of plants from all parts of the world in the botanic garden. He sent trained collectors to many countries overseas, bombarded ministers and government officials with letters of instruction, sometimes almost of command, and gave freely of his time, energy, and money to this end. It is said that about seven thousand plants from abroad new to cultivation in England were thus introduced to Kew during the reign of George III. Among the 'Kew collectors' of this period were Francis Masson (1741–1806), who collected in South Africa, the West Indies and North America; David Nelson (died 1789), who visited Australia, South Africa and Timor; A. P. Hove, who was in India during 1787–89; Peter Good (died 1803), who sent plants from India and Australia; George Caley (died 1829), who went to Australia; William Ker (died 1841), an early collector in China; the brilliant botanist, Allan Cunningham (1791–1839), who collected widely in Brazil, South Africa, Australia and New Zealand; James Bowie (died 1869), who sent material from Brazil and South Africa; and David Lockhart (died 1846), who went to the Congo and, later, to Trinidad. Another collector who, through Sir Joseph Banks, made important contributions to the botanical development of Kew was Archibald Menzies (1754–1842), who was surgeon and botanist to Captain George Vancouver (1758–98) in his voyage of survey (1791–95) and sent plants from Australia, South America and western North America. He introduced the monkey puzzle (*Araucaria araucana*) from Chile to Kew and was the discoverer of the redwood (*Sequoia sempervirens*) in California.

George III and Sir Joseph Banks both died in 1820, and Kew passed into a period of relative decline. No more collectors were sent out from Kew, though seeds and plants continued to be received from overseas. That the Gardens did not die as a botanical institution was largely due to John Smith (1798–1888), who was made W. T. Aiton's chief assistant in 1826 and later, under Sir William Hooker, became curator. In January 1838 the Treasury appointed a committee "to inquire into the management, etc., of the Royal Gardens". This committee consisted of Dr. John Lindley, a renowned botanist and author of "The Vegetable Kingdom", Mr. (later Sir) Joseph Paxton, and a Mr. Wilson, gardener to the Earl of Surrey. The resulting report described the collections, then at Kew, their condition, the state of the greenhouses, etc., and was extremely critical, though the report was itself criticized by John Smith in his "Records". However, the report on the constructive side pointed out strongly the need for a well-managed and well-equipped botanic garden in Great Britain. It was presented to Parlia-

Fig. 1. Part of the Herbarium, Royal Botanic Gardens, Kew

ment on May 12, 1840, and as a result the control of Kew was transferred from the Lord Steward to the Commissioners of Woods and Forests, that is, from being the personal property of the Crown to the nation. Sir William Jackson Hooker, then regius professor of botany in the University of Glasgow, was appointed director in March 1841.

Kew as we know it to-day is very largely the creation of Sir William Hooker. Apart from being a first-class botanist, he was an organizer and administrator of the first rank. When he became the first director of Kew officially appointed by the Government, the garden area in his charge was only about 15 acres, and this was divided by brick walls which he speedily had pulled down. When he died, in 1865, six weeks past his eightieth birthday, Kew Gardens extended over 250 acres. Such figures, however, are only superficial indications of what Sir William did for Kew. New plants were received from many sources, a large part of the ground was laid out afresh, the Palm House was built between 1844 and 1848, the first Museum of Economic Botany was opened to the public in 1848, the Herbarium and Library were commenced in 1853, the Lake was completed in 1856, the first flagstaff successfully erected was presented in 1861, and the Temperate House was completed in 1862. Sir William continued his botanical and editorial activities, many of which had been started in Glasgow, after his appointment as director of Kew, and he encouraged botanical research by his staff and by visitors.

Sir William's only surviving son, Joseph Dalton Hooker (knighted in 1877), had been assistant director during 1855–65 and was appointed director in immediate succession to his father. He was born, as the second son, in 1817 and was educated in Glasgow. After taking his M.D., he was appointed assistant surgeon in the Royal Navy and was gazetted to the *Erebus* which, with the *Terror*, under the command of Captain (later Sir) James Ross, visited many parts of the southern hemisphere. J. D. Hooker's large collections were later worked out by him and published in a series of important volumes dealing with the taxonomy and phytogeography of the countries explored. From late 1847 until early 1851 he was in India and for three entire years explored and collected especially in Sikkim, eastern Nepal, and the Khasia Hills. His interest in Indian botany, which culminated in the preparation and publication of "The Flora of British India" (seven volumes, 1872–97), remained with him all his life. After his appointment as director of Kew, J. D. Hooker undertook two more botanical journeys, one to Morocco in 1871 and one to western North America in 1877. The most important taxonomic work was the "Genera Plantarum" (1862–83), written in collaboration with George Bentham and containing descriptions in Latin of all the then known families and genera of seed-bearing plants. These descriptions have, as a whole, never been excelled, and the volumes are still constantly used by taxonomists. Besides his own researches, J. D. Hooker encouraged much and diverse botanical investigation at Kew, particularly in taxonomy, plant geography, and economic botany. He made numerous improvements in the Gardens. Thus, he planted the Pinetum, brought the Berberis Dell to its present condition, created the Rock Garden, planted the Holly Walk, and improved avenues and vistas. The T-range was erected in 1868–69, the Jodrell Laboratory was built in 1876, a new wing was completed for the Herbarium in 1877, a wing added to Museum I in 1881, and the North Gallery opened to the public in 1882. After his retirement from the directorship in 1885, Sir Joseph Hooker lived at Sunningdale in Berkshire and continued to edit the "Icones Plantarum" until the end of 1889 and the *Botanical Magazine* until 1902 and for two years more with assistance. He completed the "Flora of British India", and during the final years of his long life continued researches in the genus *Impatiens*, results of which were published up to the year of his death, 1911. He was buried, with his father, in the family vault in St. Anne's Churchyard, Kew.

The history of Kew during 1841–85 is almost synonymous with the biographies, during that period, of the two Hookers, father and son. It gives some idea of their tremendous activities to record that in

Fig. 2. *Victoria regia*, giant water-lily of the Amazon. The leaves attain a diameter of up to 6 ft.

the Kew Library there are 76 volumes (many large) of Hookerian correspondence. Administration had its difficulties, and the famous controversy in 1872 between the director and his official superior, the First Commissioner of Works, A. S. Ayrton, led to debates in both Houses of Parliament, the publication of a 'bluebook', and finally the placing of the authority of the director of Kew on a firmer basis.

J. D. Hooker was succeeded in the directorship by his son-in-law, William Turner Thiselton Dyer (the last two names became hyphened about 1891). Dyer was particularly keen on making Kew the botanical centre of the British Empire, especially as regards plant taxonomy and economic botany, and he did much in this direction by stimulating the preparation of Colonial floras. A new wing of the Herbarium was built in 1902, and the *Kew Bulletin of Miscellaneous Information* (afterwards the *Kew Bulletin*) was started in 1887. The original "Index Kewensis" was published in 1895. Improvements were made in the Gardens, and houses for alpine plants, filmy ferns and pitcher plants were erected. The Queen's Cottage Grounds were given in 1898 by Queen Victoria to Kew to commemorate her Diamond Jubilee, and Cambridge Cottage and its walled garden were added in 1904. On April 1, 1903, the Royal Botanic Gardens, Kew, were transferred from the Office of Works to the Board of Agriculture and Fisheries (now the Ministry of Agriculture, Fisheries and Food).

Thiselton-Dyer resigned in December 1905 and was succeeded by Lieut.-Col. David Prain, who steadily developed the scientific activities of Kew until 1922, in spite of setbacks during the First World War. Dr. A. W. (later Sir Arthur) Hill was director from 1922 until his tragic death in a riding accident in 1941. After a short interval, Prof. E. J. (later Sir Edward) Salisbury was appointed to the directorship and retired in 1956, and the present director is Dr. G. Taylor, formerly keeper of the Department of Botany, British Museum (Natural History). During the past fifty years, additions to Kew include a fine new wing to the Herbarium (built in 1931), enlargements to the Rock Gardens, the Sherman-Hoyt House for Succulents, a Chalk Garden, a Clematis Wall, a modern Australian House, and new accommodation for the Orchid Collection. Many of the greenhouses have been renovated or reconstructed since the Second World War, notably the Palm House. The old Orangery, designed by Sir William Chambers and erected in 1760–61 and long used as a museum for timber exhibits, etc., is being converted to serve again its original purpose.

Since 1841 the Royal Botanic Gardens, Kew, have been a government institution serving an international function—the advancement of botanical science. From the nature of botany, certain divisions of the subject can only be studied adequately at a large central institution, and it is these that are the special concern of Kew. In particular, the three branches of plant taxonomy, phytogeography, and economic botany require large collections of specimens, a first-class library and facilities for wide contacts overseas. Kew provides these conditions probably more adequately than any other institution in the world, both to members of the staff and to very many students from all countries who visit Kew for longer or shorter periods. In addition, a great deal of assistance is given to correspondents in answering a very large variety of questions, in providing material for research, and in loaning specimens to recognized botanical institutions. The work of Kew is not limited to the three branches of botany mentioned above, but it is even more essential to record that these branches themselves are considered at Kew along modern lines to include or to have contact with most of the specialized subdivisions of botany that are dealt with in other institutions. Of the large collections at Kew that are available for research one may mention the living plants in the Gardens, with more than 45,000 species, the Herbarium of dried specimens exceeding 6,000,000 sheets including more than 200,000 types, the sets of microscope slides at the Jodrell Laboratory, and the extensive series of samples of economic products (timbers, fibres, foods, drugs, etc.) in the museums.

The continued contribution of Kew to botanical research may be briefly indicated by reference to publications. The long series of floras produced at Kew include the "Flora Australiensis", the "Flora of British India", the "Flora of Tropical Africa", the "Flora Capensis", the "Flora of West Tropical Africa" (now being revised), and many smaller works. In course of preparation, and in part publication, are the "Flora of Tropical East Africa", the "Flora of Cyprus", the "Flora of Iraq" and (in collaboration) the "Flora Zambesiaca". Assistance is also being given in the preparation of the "Flora of Trinidad and Tobago" and the "Flora Malesiana". The *Kew Bulletin* is now published with three parts to an annual volume and contains papers, mostly by members of the staff, on a variety of botanical subjects. The "Index Kewensis", one of the most useful works to systematists concerned with vascular plants, is compiled at Kew and supplements are now published quinquennially. The "Icones Plantarum" consists of black-and-white figures, with descriptions and other information, of plants preserved in the Kew Herbarium, and is published by the Bentham-Moxon trustees in parts at intervals. The *Botanical Magazine*, the oldest botanical and one of the oldest scientific publications still continuing, was commenced in 1787 and has been connected with Kew for more than a century. It has coloured figures prepared from living plants grown at Kew or in other British gardens with accompanying text giving a full descriptive and general botanical account of the plants depicted. It is prepared at Kew and published by the Royal Horticultural Society as a biennial volume of four parts. In addition to the floras and periodicals enumerated, members of the Kew staff contribute scientific papers to many scientific journals and societies and have published various independent books on a wide range of subjects including taxonomy, plant anatomy, phytogeography, economic products, ecology, and genetics.

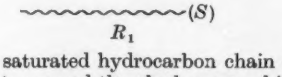
In this brief sketch of the history and functions of Kew emphasis has been placed on the botanical activities of the institution. The educational and recreational value to the public are also very considerable. The Gardens are interesting and beautiful at all seasons of the year, above all in the months of May and June. In 1958, a 'bad weather' year, the number of visitors passing through the turnstiles was 946,499, and though many of these came for a 'day's outing', a high proportion gained knowledge concerning the wealth of form, colour and behaviour of a great range of plant life and of the use of trees, shrubs, and herbs in man's economic and æsthetic life. Kew's contribution to the welfare of the nation, the Commonwealth, and the world cannot be precisely measured in monetary or any other terms, but it is certainly very great.

THE MECHANISM OF DETERGENCE

By Dr. A. S. C. LAWRENCE
Department of Chemistry, University of Sheffield

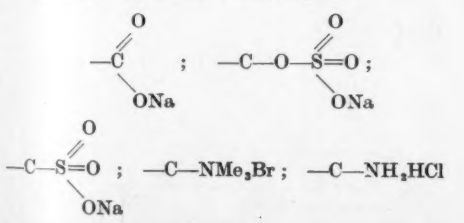
FOR nearly forty years since the discovery of orientated monomolecular films, detergent action has been described in terms of adsorption, wetting angles and reduction of surface free energy. Substrate and dirt have been regarded as inert non-polar surfaces on to which the soap is adsorbed with the hydrocarbon chain inwards and the polar group outwards to give wetting by water. As generalizations, the first is dubious and the second untrue. This general theory has no place for specificities of soaps for soils or substrates, nor can it explain the nature of the actual detachment and breaking up of dirts of sticky consistency and strongly adherent character. Indeed, some writers have been careful to say that there is no justification for saying that detergence is a spontaneous process. Nevertheless there is in the detergence of polar dirt a spontaneous process of penetration of soap and water into the dirt, followed by peptization. This process has nothing to do with surface forces, but results from cryoscopic forces and diffusion processes in the ternary soap/water/polar dirt system. This new picture of the mechanism of detergence arises from the following considerations.

It is now known that a large number of organic substances, especially fatty ones, are soluble in aqueous soap solution even though insoluble in water, and that substances partially miscible in water have their mutual solubilities greatly increased by addition of soap. By 'soap' I mean any substance which has the properties of soap; that is, a substance which in aqueous solution is surface active, froths, emulsifies oils and has detergent action: a definition which includes the substances variously described as synthetic detergents, syndets, surface-active agents, surfactants and even, in the horrible advertising jargon, 'soapless soaps'. Those most studied are of the type:

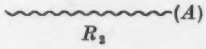


R_1 (S)

where R_1 is a saturated hydrocarbon chain of 12, 16 or 18 carbon atoms, and the oleyl soaps, which have a C_{18} chain with a double bond in the 9 : 10 position, and the *cis* configuration, which gives them solubility corresponding more nearly with the C_{12} saturated chain than the C_{18} chain. S represents:



I am not concerned now with non-ionic soaps, where the picture is more confused. The organic substances which we have studied most thoroughly are the aliphatic acids, alcohols, and amines, which may be written generally as:



R_2 (A)

A benzene ring can be included in the R_2 chain and more complex substances such as cholesterol show similar solubility effects. These are all substances of the type named by Winsor 'amphiphilic'[1]; that is, substances containing both a polar water-soluble group and a water-insoluble group of size sufficient to confer hydrophobic properties—a structure which ensures that the two parts of the molecule always have opposed solubility tendencies. Winsor includes the soaps in the amphiphilic class, but I prefer to keep them out of it and call them soaps.

Ternary Soap/Water/Amphiphile Systems

At first sight, the changes observed when an amphiphile is added to aqueous soap solutions of various concentrations seem quite irrational, and phase analysis appears to be impossible because of the very high viscosities encountered. Once, however, the presence of a ternary liquid crystalline phase existing over a wide range of concentrations was established[2], study of phase equilibria became possible, and the full triangular diagrams have shown a remarkable consistency independent of the nature and charge of S and A, or of R_1 and R_2 provided R_2 contains five or more normal carbon atoms (Fig. 1)[3-5]. The diagram consists essentially of five areas (Fig. 1); at the top, at high concentrations of soap, solid soap exists; at the bottom, below about 10 per cent soap, there are two liquids, sometimes as separate phases or, with higher values of R_2, as a system in which the aqueous isotropic phase is enclosed in membranes of liquid-crystalline phase and which defy mechanical separation. This is not an emulsion although it resembles one to casual visual inspection. At the water-rich corner, L_1 is isotropic solution; this is the region of McBain's 'solubilization'. At the corner rich in organic component another isotropic liquid, L_2, exists; this is not formed merely by turning the micelles in L_1

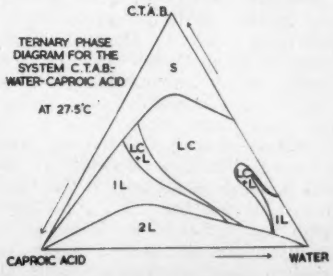


Fig. 1. Typical phase diagram for soap/water/amphiphile system. The diagram is for hexadecyltrimethylammonium bromide, water and hexanoic (caproic) acid at 27·5° C.

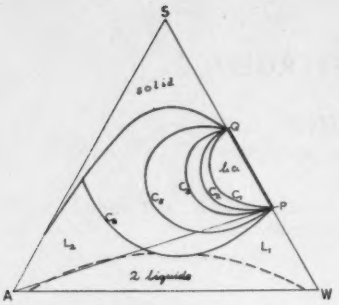


Fig. 2. Simplified diagram of the effect of chain-length of amphiphile (R_2) upon the phase boundaries of the ternary liquid-crystalline phase

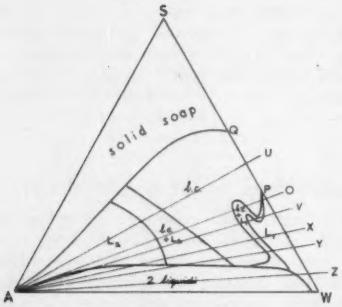


Fig. 3. Phase diagram for the system: sodium dodecyl sulphate/water/hexanoic (caproic) acid. Tie lines are shown to indicate the different sequences of changes when caproic acid is added to different initial concentrations of soap in water at 25° C.

inside out; they are reversed so as to become lipophilic in L_2 but, as they contain water, they are emulsions of truly colloidal dispersion[6], as opposed to the classical emulsions the dimensions of which place them far outside the range of true colloids. L_2 is the region of formation of the commercial 'soluble oils' when hydrocarbon is added as a fourth component. LC is the 100 per cent (smectic) liquid-crystalline phase flanked on either side by an area of $LC + L_1$ or L_2.

When R_2 is a 1, 2 or 3 carbon-atom chain or if the amphiphile is phenol or cresol, no two-liquid area occurs, L_1 and L_2 become continuous and extend to the base of the triangle. The liquid-crystalline (*l.c.*) phase, however, still exists as shown in Fig. 2. From this and from Fig. 1 it is clear that the *l.c.* area has, as base on the soap–water side of the triangle, the range of concentrations, PQ, over which the binary soap/water system is liquid-crystalline. This has been shown most clearly by Dervichian[7] in his potassium hexoate/water/methanol system; it is also clear why, starting from isotropic soap solutions, and adding the organic amphiphile, we do not observe liquid-crystalline phase when R_2 is a chain of less than five carbon atoms; the ternary liquid-crystalline phase exists but it is necessary to start with a soap solution which is already in the binary liquid-crystalline phase (tie line AP, Fig. 2 and AU in Fig. 3). If in Figs. 1, 2 and 3 we choose a concentration of soap in water and add amphiphile to it, we travel along a tie-line from that initial concentration to the 100 per cent amphiphile corner; the great variety of phase changes observed for initial soap concentrations of U, V, X, Y and Z are indicated in Fig. 3, which represents the phase diagram for sodium dodecyl-sulphate with caproic acid (amphiphile) and water. We see that we shall get different changes starting from concentration V of hexadecyltrimethylammonium bromide and of sodium dodecyl sulphate in Figs. 1 and 3. Again, if we wish to make a soluble-oil type such that no separation into two liquid phases occurs, then the minimum soap concentration is given by AO, the tie line from A tangential to the top of the two-liquid phase area.

This picture applies to all ionic soaps and amphiphiles regardless of the nature of the radicals A and S or of the sign of their charges. We have made a number of fatty alcohol/ethylene oxide-chain soaps which are interesting in so far as their solubility depends upon hydrogen bonding between the ethylene oxide chain and water. The aqueous solutions show a lower consolute temperature; that is, the soap and water separate into two liquid layers when the aqueous solution is heated. Addition of amphiphiles will lead to competition for hydrogen bonding and the case is different from that of ionic soaps. I suspect also that all these soaps are in fact true soaps solubilizing some of the lower water-insoluble polymers containing one, two or three ethylene oxide chains; the stepwise addition of ethylene oxide to form the polymer chain ensures that with an average number of, say, 6 ethylene oxide chains there must be some with more or some with fewer chains; the latter, together with any unreacted ROH, will therefore constitute amphiphiles already present in the soap solution, and extreme caution is necessary in interpreting the results of further deliberate addition of other amphiphiles.

Temperature Effects

With the higher homologues of the aliphatic amphiphiles, a lower temperature limit to the existence of the ternary liquid-crystalline phase is set by the point where the amphiphile crystallizes out; when this is present in excess, it freezes out at 2–3 deg. C. below its melting point. At more nearly equal ratios of soap and amphiphile, however, there is a large depression of freezing point such that the

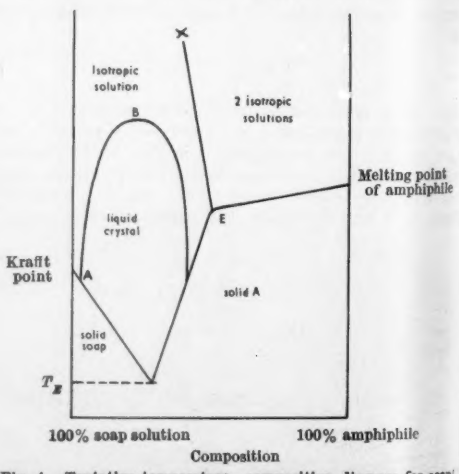
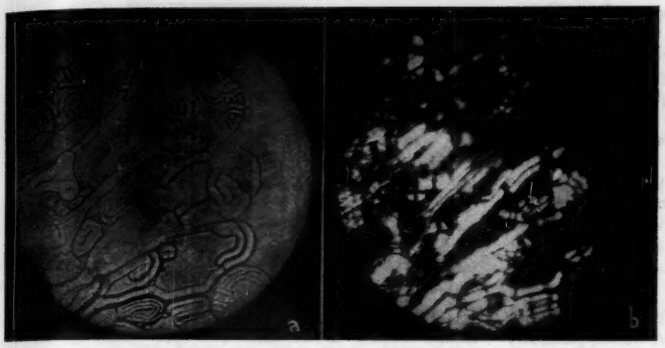


Fig. 4. Tentative temperature – composition diagram for soap/water/amphiphile systems for any selected soap–water concentration from the minimum required to avoid the two-liquid layer region up to the maximum at which the soap solution becomes liquid crystalline



Nicols parallel (×50) Nicols crossed

Fig. 5. Myeline figures formed by penetration of water into an equimolecular mixture of sodium laurate and oleic acid; after about 10 min. at 20° C.

liquid-crystalline phase is stable at room temperature. Fig. 4 shows the temperature – composition diagram for such systems, treating them as binary mixtures with soap and water as one component and the amphiphile as the other; the Krafft point of the aqueous solution is taken as a melting point, while the other melting point is, of course, the orthodox one. It applies to all soap/water ratios between the minimum of about 10 per cent required to prevent formation of the lower two-liquid area and the upper limit at which the binary soap/water system becomes liquid crystalline. It will be seen that this is only a partial limitation involving the movement of the left-hand rising boundary of the *l.c.* phase from *A* to the $(S + W)$ axis; the low-temperature eutectic part of the diagram below the Krafft point being unaffected.

If the lower axis of the graph represents room temperature, then on the left we have solid soap plus solution; on the right, solid amphiphile plus solution; and in the centre ternary liquid-crystalline phase: this is found even with cholesterol as amphiphile which, in spite of its high melting point (147° C.), persists at 0° C. The upper temperature limit of the liquid-crystalline phase is usually above 100° C. The other cases in which either the melting point of the amphiphile or the Krafft point is below 0° C. have been described elsewhere[3]; also the case where both are below 0° C. and the first solid to separate out is ice.

Penetration Experiments

Fig. 4 suggests that the ternary liquid-crystalline phase should form spontaneously at room temperature by mixing the components. This can be shown to occur in two ways, both being observed most conveniently by the polarizing microscope. First, by placing a very small piece of soap plus amphiphile, as solid (or paste if the latter component is liquid), pressing a cover-glass down gently and then flooding with water. After 5–10 min., a crop of myelinic tubular forms is seen under a magnification of 50; they are best seen with the nicols parallel (Fig. 5*a*), although with crossed nicols the myelines are seen to be strongly birefringent close to the original material and becoming less and less so as they become more elongated. Finally, the outer part breaks up by dissolving to form an isotropic solution, L_1 (Fig. 5*b*). The second way is by immersing the solid amphiphile in soap solution; here also spontaneous formation of the ternary liquid-crystalline phase occurs but with two differences: the tubular myeline-like protuberances shown in Fig. 6 are sausages of liquid crystal and not tubular like the true myelines which have solution inside, and are elongated because the membrane containing them is a smectic liquid crystal with the axes of the long-chain molecules normal to the axis of the myeline so that extension can therefore take place only in a telescopic manner.

The second difference is that there is a sharply defined minimum temperature above which penetration of the soap solution into the solid amphiphile occurs (Fig. 7). Below this temperature (T_{pen}) only very slow formation of isotropic solution L_1 is

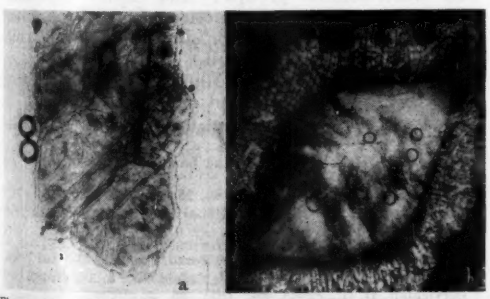


Fig. 6. Penetration of 15 per cent sodium dodecyl sulphate solution into solid stearic acid at 50° C.; (*a*) initial membrane in white light, and (*b*) after 6 min. in polarized light (× 20)

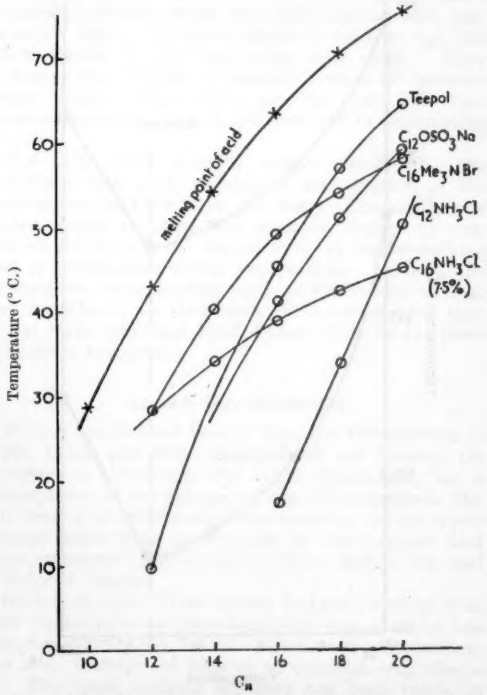


Fig. 7. Variation of temperature of penetration with chain-length for homologous fatty acids

observed. T_{pen} is several degrees below the melting point of the pure fatty amphiphile, and in the case of the eutectic curve of stearic and palmitic acids falls on a eutectoid curve with its minimum at the same composition but at a lower temperature (Fig. 8). Clearly T_{pen} should be identical with T_E, the eutectic temperature in Fig. 4. It is of interest in connexion with the ease of this penetration that the liquid crystalline growths are around the periphery of the crystal where polar groups are accessible and not on the large surfaces of the plates which are the terminal methyl groups.

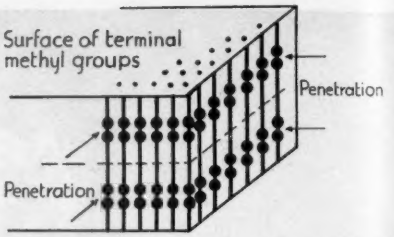


Fig. 9. Plate-shaped crystal of fatty amphiphile showing that the polar groups are accessible at the edges but not on the large flat faces of the plates

Detergence

It then occurred to me that these fatty amphiphiles are dirt—polar dirt it is true, but most dirt is partially polar—and that Fig. 7 is a picture of the spontaneous action of a detergent penetrating and peptizing dirt; that the triangular diagrams are soap/water/dirt phase diagrams and that Fig. 4 is the diagram of the temperature changes involved in detergence. Myeline formation has been reported in detergence by Stevenson[8] and also by Harker[9].

It may be objected that the process described requires a concentration of soap much larger than that normally used in detergence, but the objection is not valid because there will be a close-packed adsorbed monolayer of soap at the solution–dirt interface. At the point of attack the soap will always be a condensed phase and the interaction is of zero order with respect to the bulk concentration of soap. In fact, penetration occurs with solutions as dilute as 0·1 per cent.

Any line drawn from the solid stearic acid (shown in Fig. 6*b*) out into the isotropic solution is a tie line on the triangular phase diagram (perhaps not on a linear scale) from the 100 per cent amphiphile corner to the appropriate soap concentration; for example, *AO* in Fig. 3. We pass from solid amphiphile through fluid L_2 solution, very viscous *l.c.* into fluid L_1 solution; the dark isotropic ring around the stearic acid is the L_2 solution and, if the cover glass is disturbed, the *l.c.* phase is broken up, and floats away. Soap diffuses in again and builds up more *l.c.* and reduces the rate of diffusion of soap inwards. The gelatinous *l.c.* membrane is always separated from the 'dirt' by fluid L_2 phase; this is the reason why all washing requires agitation.

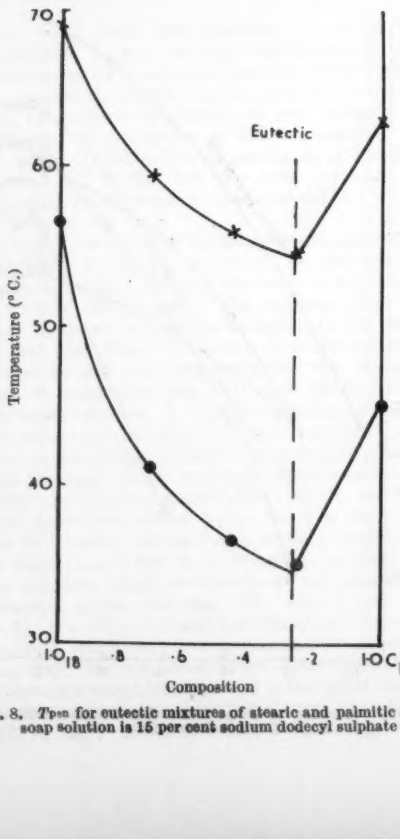


Fig. 8. T_{pen} for eutectic mixtures of stearic and palmitic acids; soap solution is 15 per cent sodium dodecyl sulphate

As described, fatty acids and alkanols are readily penetrated at temperatures greater than T_{pen}. Glycerin monolaurate was also penetrated but not the dilaurate. Addition of fatty acid or alcohol to 'Nujol' and paraffin wax showed that penetration in the former required a minimum concentration of about 10 per cent, whereas the wax needed at least 20 per cent.

An account has been presented of a mechanism of the process of detergence in terms of cryoscopic forces; the existing theory in terms of surface tension and free surface energy has always ignored this fundamental process. Wetting-angle phenomena are not affected by the new picture. Two outstanding problems, that of the very large temperature coefficient of efficiency of detergence and the rather mysterious one of 'redeposition', may well become explicable; the first as an increased rate of diffusion and the second by the fact that rinsing is a dilution so that we drop almost to the bottom of the ternary diagram into the two-liquid area, and, in this, the soap is in the organic phase: that is to say, the dirt is taking the soap out of the water.

The mechanism postulated here suggests that detergence is not brought about by free surface energy phenomena but by cryoscopic forces only. Ternary eutectic formation is by the water and the two water-soluble groups; the liquid crystalline properties result from the close-packing of the water-insoluble hydrocarbon 'tails' attached to the polar groups of soap and fatty soil.

I am indebted to Dr. T. Malkin for pure specimens of glycerin monolaurate and dilaurate.

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[2] Hyde, A. J., Langbridge, D. M., and Lawrence, A. S. C., *Farad. Soc. Disc.*, **18**, 239 (1954).
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DEVELOPMENTS IN PHOTOELASTICITY

COMMEMORATION AT UNIVERSITY COLLEGE, LONDON

A JUBILEE commemorative exhibition of photoelasticity has been held in University College, London. With a lecture delivered on April 15 by Colonel H. T. Jessop, special lecturer in photoelasticity in the College and sometime research assistant to Prof. L. N. G. Filon, it recorded the progress made in this method of stress exploration in the fifty years since its two most formidable protagonists, Profs. Coker and Filon, began their uneasy but extremely profitable partnership; E. G. Coker was the Kennedy professor of engineering and L. N. G. Filon was the Goldsmid professor of applied mathematics in the College.

In 1909 Prof. Filon had been working for some years on the optical phenomenon of stress-induced birefringence, which had remained a laboratory curiosity since its original discovery by Brewster in 1816. While an undergraduate at University College, he had become interested, too, in the mathematical theory of elasticity as a result of his contact with Karl Pearson.

By 1909 Prof. Coker had also been attracted by the subject and his interest was that of a mathematically uninhibited engineer. He wanted to know the stresses in bodies for the purposes of design studies. He was introduced to photoelasticity by Silvanus Thompson and, in a four-year period of collaboration, these two men proposed a series of stress investigations using celluloid models of structures having engineering significance.

Coker seems to have been the first to realize the practical engineering significance of stress-induced birefringence, and his proposals precede similar work of Mesnager (who used glass) by three years. Coker's use of celluloid models was largely intuitive, and Filon pointed out the many theoretical questions that it begged. This brought the two men together, though no details are available of the first meeting in 1909. They clearly had very different approaches to the subject; even so, their subsequent collaboration lasted until Coker's retirement twenty-seven years later. Furthermore, the text-book which bears their names has no equal, and is, for those interested in photoelasticity, what Love's treatise has been for elasticians.

The Exhibition of Apparatus

The early exhibits show the differing interests of these two pioneers. Filon's experiments and apparatus illustrate his concern with the optical behaviour of materials, and in particular glass, under simple and calculable states of stress. Coker's apparatus shows how he wanted the practical answers that could be obtained from each experiment, and he seems to have taken it as axiomatic that the elastic stresses—and in particular the maximum values of stress—were the significant quantities. Coker concerned himself with the purely two-dimensional problem of photoelasticity with three unknown quantities at each point. For his purposes, the directions of the principal stresses were obtained from isoclinics in a plane polariscope, the difference between the two principal stresses was deduced from the isochromatics in a circular polariscope, and their sum was found by the measurement of lateral strains. To this limited extent Coker could have claimed that he had completely 'solved' the problem of two-dimensional photoelasticity—indeed, the claim has often been made for him.

Filon seems to have distrusted the lateral strain measurements, although he verified their validity for a state of 'generalized plane stress'. He relied instead on the differential equation of equilibrium to give him the extra information which permitted the principal stresses to be separated. But for Filon's approach, the measurements which could be taken were not sufficiently accurate to justify calculation of their derivatives. This led him to consideration of stress trajectories which gave a curvilinear co-ordinate system, from which the so-called Lamé–Maxwell equations could be obtained. The errors of calculation were then mainly included in a single estimate of a radius of curvature.

Only a few pieces of Filon's apparatus survived the bombing in the Second World War. His first experiment on a glass beam in bending still exists, but the rest of the original apparatus has been lost. Two of his early experiments on dispersion and a calibration experiment on glass have been re-assembled by Colonel Jessop, who was Filon's research assistant when the experiments were performed. Many of Coker's ingenious loading rigs and other pieces of his apparatus still exist. They illustrate his interest in stressed bodies of complex shape which, while they are far removed from mathematical abstraction, are common in engineering practice.

Not only were 'museum pieces' displayed; the exhibition also gave an idea of the progress of the photoelastic method in recent years. The exhibits of recent origin revealed the amazing degree of confidence which is now placed both in model-making and in subsequent stress exploration. In fact, the method has been applied (both at University College and elsewhere) to structures of a complexity that would have surprised Prof. Coker, even in his most optimistic moments.

Recent Developments

During the Second World War, the laboratories of Profs. Coker and Filon disappeared, one literally (as a result of bombing), the other figuratively as a consequence of the rigours of war-time academic life. The revival of interest in photoelasticity in University College since then owes much to the support and encouragement of Prof. H. J. Collins and to the zeal of Colonel Jessop.

Both Coker and Filon appear to have believed that their exploitation of photoelasticity was more or less complete. But here, for once, they were quite wrong, and it is, perhaps, of interest to consider why this is so. The most striking advance has been made in three-dimensional work. This extension has been made possible by several factors. Of these, the

development of a really suitable synthetic resin is a logical extension of Coker's step from glass to celluloid. With the appearance of modern epoxy casting resins, and in particular of 'Araldite *B*', practical materials have become available in adequate sizes; they possess good elastic behaviours, negligible creep, and sufficient optical sensitivity to be useful. With these new materials has come the stress-freezing technique—now more than twenty years old; this opens up an almost foolproof method for the analysis of solid models. By subjecting a model to a heating and loading cycle, it can be persuaded to 'remember' its loading system, and to retain an optical pattern which is equivalent to an elastic system of stress. The solid model can then be sectioned without disturbance of the optical effect, and a three-dimensional analysis can be made. The possibility of doing this was noted in Germany in 1935, but was apparently not known to Filon or to Coker (who was, by then, a sick man). A similar effect had been noted in glass at University College by Dr. F. C. Harris, although its implications for three-dimensional work were not recognized.

It is interesting to note that, with this development, one returns to the original difficulties of measurement which faced Coker. However, his primitive methods have been superseded by the techniques due to Tardy or Sénarmont. Sufficiently accurate measurements can now be made with strains in the model which are not more than ten times the strain in the prototype.

The interpretation of the results found from a model in terms of the corresponding full-scale structure in a different elastic material, and the effect of a tenfold increase in magnitude of strain in the model, was discussed in a review of the present state of knowledge. This was given at University College in two lectures on modern aspects of photoelasticity by Mr. C. Snell. He argued that although the position is not entirely clear, there are good theoretical and experimental reasons for transferring the stress pattern without significant change from model to prototype.

The problem of thermal stressing was exemplified in the exhibition, and the experimental difficulties that have yet to be overcome were mentioned. Dynamic stressing was also illustrated, with a demonstration that problems of dynamic elasticity can sometimes be studied by photoelastic means. In particular, periodic excitation can be investigated in this way unless the periodic time is too short for stroboscopic techniques to be adequate.

The use of optically sensitive layers to show the strain patterns in metals beyond the elastic range is probably the most recent development in photoelasticity. This technique was illustrated in two exhibits and is undoubtedly a promising new departure. Perhaps not so promising—but by no means without interest—was some apparatus (of recent origin) for investigating the optical behaviour of a moving fluid.

Photoelasticity in Engineering

These, and many other, exhibits leave no doubt about the future of photoelasticity. It has been put to use time and time again, particularly in the aircraft industry, where the economical use of material is vitally important. Now it seems clear that the methods will be widely employed in design work on nuclear engineering projects.

Historically, our knowledge of the stress-optical effect is of Scottish origin, the earliest investigations having been made by Brewster and, later, Maxwell. But practical stress exploration undoubtedly grew up in University College, London. It is most unlikely that any other institution could arrange, from its own resources, an exhibition covering so wide a range of productive work within photoelasticity—even if the earlier exhibits of purely historical interest are disregarded. This is a direct outcome of the collaboration of Profs. Coker and Filon—an engineer and a mathematician—and of the subsequent efforts of a small group of workers under the leadership of Colonel Jessop and, since his retirement, Mr. Snell.

R. E. D. Bishop

OBITUARIES

Dr. Paul Chabanaud

Dr. Paul Chabanaud, Chevalier de la Légion d'Honneur, well known for his researches on fishes, died on February 27 at the age of eighty-two.

Although he had been from childhood a keen amateur naturalist, Chabanaud began by preparing himself for a career in the law, but he considered this to have been a false start. Soon after the beginning of the First World War he was declared medically unfit for the Armed Forces and became a voluntary assistant to Prof. Roule at the Museum of Natural History in Paris, classifying and preparing exhibits from the collections of reptiles and amphibians.

In September 1919, Chabanaud received the blessing of the professors of the Museum to fulfil his great ambition of a collecting trip to tropical West Africa. Apart from free passage to French Guinea and free rail travel within the territory, the only financial assistance he received was a grant of ten or twelve hundred francs. He spent about eight months in the field, covering about 1,200 km. on foot, and brought back valuable collections of arthropods, reptiles, amphibians and fishes, as well as considerable ethnological material.

It was only on his return from this voyage that Chabanaud sought any remuneration for his scientific work, and in 1920 he was appointed Préparateur à l'École des Hautes Études, working in the Laboratoires des Pêches et Productions Coloniales d'Origine Animale. From this time his main studies were in ichthyology, and it was in this field that in 1936 he obtained his doctorate, with a thesis on the morphology and phylogeny of the flatfishes (Heterosomata). This penetrating study confirmed the groupings recognized by Tate Regan in 1910 and 1929, and demonstrated a better understanding of the details of flatfish structure than had been possessed by any of his predecessors. His interpretation of this classification, however, was unique. He agreed with Tate Regan that *Psettodes* was a little-modified percoid fish, diverging less from the symmetrical form than any other flatfish. But whereas Tate

Regan looked upon this genus as a survival from the base of the flatfish stem, to Chabanaud it was the latest departure from the path of percoid symmetry, and the more specialized soles and flounders were the modern twigs of earlier asymmetrical branches of the pre-percoid trunk. The fossils so far do not help to resolve this difference, for as Chabanaud himself was to describe, the main groups were already differentiated in the Upper Eocene.

The majority of Chabanaud's numerous publications after 1936 were on details of the morphology and classification of these asymmetrical fishes, in which he found an endless fascination. He applied to them a terminology of his own, which, in spite of its logical classicism, is unlikely to supersede current usage, which has 'just growed'. It was his ambition to write the second volume of the monograph of the flatfishes begun by J. R. Norman, but he began too late in life to analyse the many samples of soles and tongue-soles available to him and never arrived at a satisfactory synthesis.

Up to the eve of his last illness, Chabanaud would leave his flat in the Rue des Écoles at 8 a.m. and walk, painfully of late, to the laboratory in the Rue Cuvier to count more fin-rays and scales and cover more sheets with his endless tables. How I wish that in some Elysium it might be given him to draw from them the crystal truths that he was sure they would eventually yield ! He could be a charming host, and one of his most engaging accomplishments was the relating of reminiscences, plunging into parenthesis after parenthesis without faltering in the elegant use of his mother-tongue and never failing to emerge triumphantly in the end as from a series of Chinese puzzle-boxes.

He was quite unworldly, a man of child-like integrity and innocence of mind. He is survived by his second wife, with whom he spent many years of mutual devotion.

E. Trewavas

Prof. J. T. MacGregor-Morris

Prof. J. T. MacGregor-Morris died on March 18, at the age of eighty-seven. He established the Electrical Engineering Department at East London College (now Queen Mary College) in 1898, and he became the first professor of electrical engineering in this College, a position which he held until he retired in 1938.

He received his education in electrical engineering at University College, London, and, for a time, was personal assistant to Sir Ambrose Fleming. He was keenly interested in research and during the Second World War he did notable work in developing a hydrophone for submarine detection.

An important contribution to electrical engineering education by Prof. MacGregor-Morris was the manner in which during the lean years immediately following the First World War he kept research work alive in his department, despite meagre equipment and small research funds. He kept the department open on three evenings a week, and he inspired and directed the research work of students who attended on these evenings. The students were of good calibre and they did good work. Several of them have risen to important positions in the electrical industry.

Under the leadership of its professor, the Department grew steadily in prestige, and its housing and equipment improved as the years passed. A notable development was the opening of its well-known High Voltage Laboratory in 1938 with the aid of a grant from the London County Council. This Laboratory was an example of Prof. MacGregor-Morris's foresight —he was a good judge of new developments which were likely to become of major importance in electrical engineering. Another example of this was his interest in the cathode-ray tube from its earliest days. He was joint author of one of the earliest text-books dealing with these tubes.

His lectures were interesting, stimulating and unorthodox with his over-riding interest in research always intruding.

His students and particularly his research students were important personages to him. Many of them will recall with pleasure the annual 'get-together' of staff and research students at his home in Hampstead. Mrs. MacGregor-Morris always took an active part on these occasions.

He was a very active man. On most Wednesday afternoons during term time he would play a strenuous game of tennis or badminton. He was keenly interested in music and, when quite elderly, he learned to play the 'cello and became a proficient performer on this instrument. He was a devout Christian and took an active interest in missionary work.

W. J. John

Dr. John Henderson, C.B.E.

Dr. John Henderson, for seventeen years director of the New Zealand Geological Survey (N.Z. Department of Scientific and Industrial Research), died suddenly at his home on March 6. Born in Dunedin in 1880, his later education was carried out at Otago Boys' High School, then at the University of Otago and Otago School of Mines. Here he completed, in 1902, his M.A. associateship of the School and B.E. (Mining).

For the next eight years he was director of Reefton School of Mines, during which time he devoted much energy to the investigation of the geological structure of the coal and gold mines of Westland and West Nelson, his thesis on the subject gaining him his D.Sc. in 1907.

In 1911 he was appointed mining geologist to the New Zealand Geological Survey. As well as keeping in touch with mining activities in the succeeding years, and reporting on numerous mines, quarries, and mineral properties of different kinds, he also reported on the geological implications of many engineering projects, for example the Arapuni Hydro-electric Dam on the Waikato River. In addition, he carried out extensive regional surveys of the geology and mineral resources of several districts. His bulletins on Reefton, Te Aroha (with J. A. Bartrum), Gisborne and Whatatutu (with M. Ongley), Mokau (with M. Ongley) and Huntly-Kawhia (with L. I. Grange) stand as lasting contributions to the geology of the Dominion.

At the request of the Fiji Government in 1923, Dr. Henderson visited and reported on the geology and mining prospects of gold-bearing quartz lodes in Vanua Levu.

Dr. Henderson, in 1928, succeeded the late P. G. Morgan as director of New Zealand Geological Survey and almost immediately encountered all the administrative difficulties and frustrations resulting from the financial stringency of the great economic depression. Nevertheless, by unremitting work he was able to keep his staff of geologists intact, and so actively to maintain the geological effort of New Zealand. Bulletins could no longer be printed, but

the condensed reports, and the black-and-white geological maps of the annual reports of those years, have since proved their worth.

With the coming of better times and the greatly increased activities of Works, Railways and other Government Departments and public bodies, the requests for geological advice increased enormously. The consequent expansion of the Geological Survey was carried out over a wide field, including soil surveys and geophysical surveys. Each of these has now become a separate division of the Department of Scientific and Industrial Research. About this time Dr. Henderson represented the Dominion at the International Geological Congress in South Africa, when he was able to visit the great gold mines of Johannesburg and the Rand.

In addition to his official duties, Dr. Henderson wrote a number of papers on general geology and stratigraphy, published in the *Transactions of the Royal Society of New Zealand* and in the *N.Z. Journal of Science and Technology*. During his directorship he carried on the high tradition, so well implanted in Geological Survey routine by P. G. Morgan, of most careful and accurate editing of publications, especially of the bulletins. After retirement he acted as honorary editor (1946–49) of the *Transactions of the Royal Society of New Zealand*.

Dr. Henderson was of a modest and retiring disposition, avoiding publicity, but with a deep sense of public responsibility. His interests, and also his reading, covered a wide field. This gave him a vast amount of documented information always willingly and helpfully at the service of his colleagues. To the young geologist his wealth of knowledge and kindly interest provided a real source of inspiration.

Dr. Henderson was awarded, in 1929, the fellowship of the Royal Society of New Zealand, in 1935 the Jubilee Medal, in 1945 the Hector Medal and Award of the Royal Society of New Zealand, and in 1948 he was appointed C.B.E. J. Marwick

NEWS and VIEWS

New Foreign Members of the Royal Society : Prof. Melvin Calvin

Prof. Melvin Calvin, of the University of California, Berkeley, California, whose election to the foreign membership of the Royal Society was recently announced (see *Nature*, 183, 1230; 1959), started his professional career as a control chemist in industry after the completion of his doctorate at the University of Minnesota, to which he later returned as a teaching Fellow. He then spent two years at the University of Manchester, returning in 1937 to the University of California, where he has remained ever since. His early interests were in theoretical organic chemistry and the mechanism of organic reactions.

After the Second World War Calvin took over the work initiated by Ruben on the study of photosynthesis, but employing the newly available radioisotope, carbon-14. The early results were questioned by many biologists, who from their physiological studies held strong convictions about the probable mechanism of photosynthesis; but with the timely advent of paper chromatographic methods Calvin used these to exploit the powerful potentialities of tracer techniques. From then on progress was rapid and Calvin was gradually led to formulate a mechanism of carbon reduction in photosynthesis which is now universally accepted. From studies of photosynthesis he was led to postulate enzymic systems not then isolated but afterwards discovered both in animal and in plant tissues. The photosynthetic cycle of Calvin and his collaborators may be elaborated in the future but is unlikely to be changed in its essential features. He was awarded the Stephen Hales award of the American Society of Plant Physiologists in 1956.

Calvin is the co-author of an excellent text-book on theoretical organic chemistry and his lectures have stimulated both undergraduates and research workers. He has published books on the use of isotopes and on the chemistry of metal chelate compounds. His wide interests include photochemistry and general physical chemistry as well as theoretical organic chemistry.

The Editors regret that, due to difficulties in the printing industry, some sections of this issue of "Nature" have had to be curtailed

Prof. G. Domagk

Prof. Gerhard Domagk, recently elected a foreign member of the Royal Society, has devoted his life to the fight against some of the main scourges on mankind, against cancer and against bacterial infections including tuberculosis. A graduate of the University of Kiel, Domagk began his university career in Greifswald and Münster; in 1927 he became head of the Research Institute for Experimental Pathology and Bacteriology of I. G. Farbenindustrie, Elberfeld, and it is there that he has carried out his successful researches for more than thirty years. Throughout this period he has, however, kept his ties with the University of Münster, where he was made personal professor in 1932.

His discovery in 1932 that an azo dye, 'prontosil', cured streptococcal infections in mice revolutionized medical thought, which had for many years proclaimed that chemotherapy of bacterial, in contrast to chemotherapy of protozoan, diseases was a utopian aim. It is well known how this discovery led to the development of the treatment of many serious infections with a variety of 'sulphonamides'; the award of the Nobel Prize in 1939 was in recognition of this very great advance.

It is a tribute to the impetus given to chemotherapy by Domagk's discovery that when he turned to the problem of the chemotherapy of tuberculosis, there were many groups all over the world trying to tackle the same problem. So it happened that Domagk's most successful anti-tuberculous drug, isoniazid, discovered by him in 1951, was introduced into therapy independently by a group of American

workers. Domagk was also the first to direct attention to the important antiseptic properties of the so-called 'inverted soaps'.

Domagk, whose interest in cancer dates back to 1928, is now mainly interested in the possibilities of chemotherapy of cancer. As a result of his recent work on animals, some compounds of promise are at present undergoing clinical trial. For the sake of mankind it is to be hoped that the success he has had in his other fields of endeavour will not fail him in this quest.

Prof. J. H. Oort

Few astronomers have made such important contributions to the study of the structure and dynamics of the galactic system as Prof. J. H. Oort, who was recently elected to foreign membership of the Royal Society. He was among the first to recognize the importance of the high-velocity stars, interpreting the zone of avoidance of their motions in terms of the velocity of escape from the galactic system. These studies enabled him to develop Lindblad's theory of galactic rotation into the form in which it is used to-day. He developed formulæ for the effect of galactic rotation on the radial velocities and proper motions of the stars, and analysed the observational material to obtain estimates of the angular velocity and mass of the Galaxy. Another major investigation was concerned with the motions of the stars perpendicular to the galactic plane. Oort obtained a relation between the velocities of the stars and the gravitational acceleration of the Galaxy, and with the aid of models of the Galaxy he determined the density of matter in the solar neighbourhood. Since the Second World War, Oort's most important work has been his inspiration and leadership of the team of Dutch astronomers who have been investigating galactic structure with the aid of 21-cm. hydrogen radiation. It is no exaggeration to say that, under Oort's direction, the Leyden Observatory has become one of the world's leading astronomical institutions, the work of which has had, and continues to have, a profound influence on the progress of astronomy.

Prof. A. H. T. Theorell

Prof. Hugo Theorell was also recently elected a foreign member of the Royal Society. Theorell's main work at the Nobel Medical Institute, Stockholm, has been concerned with the oxidizing enzymes, especially flavoproteins, hæmoproteins and dehydrogenases, in all of which fields he has made important contributions to knowledge during a period of more than twenty-five years. He first purified the yeast flavoprotein which had been discovered by Warburg and Christian, and later he isolated it in crystalline form. He investigated particularly the nature of the union between the flavin prosthetic group and the protein part, and showed the importance of the phosphate group in this. He also was the first to crystallize several hæmoproteins, including myoglobin and the peroxidases of milk and horseradish, and studied them in detail by physical methods, developing especially the use of measurements of magnetic susceptibility for the examination of the state of the iron atom in hæmoproteins. Theorell also investigated in great detail the structure of highly purified cytochrome *c*, established that the connexion of the porphyrin with the protein was through sulphur groups, and was ultimately able to construct a model of the structure of the part of the molecule adjoining the prosthetic group. He also carried out an important detailed study of the kinetics of the alcohol dehydrogenase system, using elegant methods depending on measurements of ultra-violet absorption and fluorescence. This led to interpretations of the apparent Michaelis constants of the various reactants in terms of the rate constants of the separate steps of the catalytic process and threw a good deal of light on the mechanism of the catalytic action of the enzyme.

Institution of Mining and Metallurgy: Awards

The Institution of Mining and Metallurgy has announced the following awards: The Gold Medal of the Institution for 1958 to Dr. A. J. Orenstein, for long and distinguished service in the cause of the health and welfare of the workers in the mining industry, with particular reference to the Witwatersrand; honorary membership of the Institution has been conferred on Mr. W. A. C. Newman, in recognition of his services both to the Institution (of which he is a past-president) and to metallurgical education; on Brig. R. S. G. Stokes, past-president, in recognition of his services to the mining industry particularly in South Africa; and on Sir Alexander Fleck, in recognition of his outstanding services to the metallurgical industry; 'The Consolidated Gold Fields of South Africa, Limited' Gold Medal for the session 1957–58, to Mr. F. A. Williams, for his paper entitled "Performance Analyses of Screens, Hydrocyclones, Jigs and Tables used in recovering Heavy Accessory Minerals from an intensely decomposed Granite on the Jos Plateau, Nigeria" (*Trans. Inst. Min. Metall.*, 67); and 'The Consolidated Gold Fields of South Africa, Limited' Premium of forty guineas for 1957–58, jointly to Mr. H. H. Fraser and Mr. O. E. A. Somerset, for their paper entitled "Scientific Management Principles applied to West African Mining" (*Trans. Inst. Min. Metall.*, 67); The 'William Frecheville' Student's Prize to Mr. I. R. M. Chaston, for his paper entitled "A Simple Formula for Calculating the Approximate Capacity of a Hydrocyclone" (*Trans. Inst. Min. Metall.*, 67).

Leverhulme Research Awards, 1959

The Trustees have approved, among others, the following research fellowships and grants tenable for periods up to two years. *Fellowship:* Dr. K. R. S. Morris (epidemiologist attached to the American Foundation for Tropical Medicine, Inc., Harbel, Liberia) for a historical and epidemiological study of the movement of sleeping sickness across Africa. *Research grants:* Maurice Black (fellow and director of studies, Trinity College, University of Cambridge) to complete a study of limestone deposition in the Bahama Islands; Geoffrey Bownas (lecturer in Chinese and Japanese studies, University of Oxford) for a comparative study of urban and rural life in Shiga Prefecture, Japan; Dr. N. H. Hartshorne (reader in chemical microscopy, University of Leeds) for a critical study of the applications of polarization microscopy in chemical and biological research and practice; Dr. K. J. Ivin (lecturer in physical chemistry, University of Leeds) to study the solution properties of polymers; G. G. Lemon (lecturer in petrology and economic geology, University College of Wales, Cardiff) to study the metamorphic rocks of the County Sligo area, Eire; Mrs. Lily Newton (lately professor of botany, University College of Wales, Aberystwyth) to study marine algae, especially those with walls yielding carrageenin and algin; D. T. Whiteside (St. Catharine's College, University

of Cambridge) for an examination of the mathematical section of the Portsmouth Collection of Newton manuscripts.

A Lunar Radio Communication Link across the Atlantic

In a series of experiments carried out between May 11 and May 14 a lunar radio communication link was successfully established between the Jodrell Bank Experimental Station in Cheshire and the Air Force Cambridge Research Center at Bedford, Massachusetts. A 1-kW. frequency-modulated transmitter working on 201·00 Mc./s. was used with the 250-ft. steerable radio telescope at Jodrell Bank. In the United States an 84-ft. radio telescope was used with receivers of various bandwidths from ± 2·5 to ± 10 kc./s. Similar receivers were also used on the telescope at Jodrell Bank for monitoring the local lunar returns. The aerial systems were circularly polarized in order to avoid the deep fading caused by Faraday rotation in the ionosphere. A series of experiments lasting for about 40 hr. included the study of the transmission of audio tones up to 15 kc./s., the transmission of messages by slow Morse, and tests of the intelligibility of speech and quality of music. The tones and Morse were received in the United States without difficulty. Speech as received back at Jodrell Bank was nearly always clear and intelligible, but only occasionally so in America because of the low signal-to-noise ratio of their equipment.

Although trans-Atlantic reception of the lunar radar pulses transmitted from Jodrell Bank was reported in 1958, the recent experiments are believed to be the first to demonstrate the feasibility of a true lunar communication link. The commercial interest in long-distance transmission secure from ionospheric and terrestrial interference will undoubtedly stimulate further developments in this field. Transmitters of somewhat greater power working in the microwave band, where the potentialities of low-noise maser or parametric receivers can be realized, should enable the size of the radio telescopes to be reduced, and at the same time provide sufficient signal-strength to ensure speech intelligibility under all conditions. This work was carried out as part of a joint programme between Jodrell Bank and the Air Force Cambridge Research Center, but the early realization of the successful lunar link was made possible by the interest of Pye Telecommunications, Ltd., which provided the transmitter and receivers.

Creation of the College of Technologists

The establishment of the College of Technologists was announced on May 20 by the Governing Body of the National Council for Technological Awards, the chairman of which is Lord Hives. The College will administer the award of Membership of the College of Technologists (M.C.T.) which the National Council proposes to create as a qualification higher than the Diploma in Technology. The College of Technologists will operate within the framework of the National Council for Technological Awards and will be served by the same administrative staff. A Board of Scientific and Industrial Studies is to be responsible for the academic and industrial aspects of the administration of the award. The governing body of the National Council has met as the Court of the College and the following appointments to the Board were made: Sir Arnold Hall, managing director, Bristol Siddeley Engines, Ltd. (*chairman*); Mr. A. W. J. Chisholm (head of the Department of Mechanical Engineering, Royal Technical College, Salford); Dr. H. O. J. Collier (director of pharmacological research, Parke Davis and Co., Ltd.); Dr. J. W. Cook (vice-chancellor, University of Exeter); Prof. P. V. Danckwerts (professor of chemical engineering science, Imperial College of Science and Technology, London, and Shell professor-elect of chemical engineering, Cambridge); Prof. E. Giffen (professor of civil and mechanical engineering, Queen Mary College, London); Dr. A. A. Griffith (research consultant, Rolls-Royce, Ltd.); Sir Stanley Harley (managing director, Coventry Gauge and Tool Co., Ltd.); Prof. A. J. Murphy (principal, Cranfield College of Aeronautics); Mr. P. E. Sleight (head of the Civil Engineering and Building Department, Brighton Technical College); Dr. G. B. B. M. Sutherland (director, National Physical Laboratory); Dr. J. S. Tait (principal, Northampton College of Advanced Technology, London); Dr. J. Topping (principal, Brunel College of Technology); Dr. R. C. G. Williams (chief engineer, Philips Electrical, Ltd.).

In addition to the members listed, the Court intends to appoint to the Board one more member of the teaching staff of a College of Advanced Technology. A further statement will be issued as soon as possible giving the details which will be needed for the submission of applications for registration as candidates for membership of the College of Technologists.

Jobs for School Leavers

Interesting proposals for dealing with the 'bulge' have been put forward by John Wellens, an industrial training consultant. This year some 900,000 boys and girls will reach the age of fifteen and in 1962 the figure will rise to 1,000,000. As at present organized, industry and commerce are quite unprepared to absorb the considerable extra number of school-leavers who owe their existence to increased birth-rate during and immediately after the War. The training facilities available for those that can be absorbed are inadequate by the most elementary of standards. To deal with the situation, Wellens suggests that a national industrial training agency should be formed, responsible to the Minister of Labour and National Service, and that the existing Industrial Training Council should act as an advisory body to the Agency ("The Bulge: Its Industrial Impact. A National Plan". John Wellens Organization, 24 Morsley Road, Grasscroft, nr. Oldham. 5*s*.). Details of the way in which the agency would work are elaborated as well as the main lines it should follow. It is doubtful whether the somewhat impractical proposals will command any wide support.

Government Support for Research in Water Supply

The Department of Scientific and Industrial Research is to give financial support to the Water Research Association. The council of the Association has been strengthened by the appointment of Mr. J. E. Beddoe, assistant secretary, Water Division, Ministry of Housing and Local Government, of Dr. B. A. Southgate, director, Water Pollution Research Laboratory, and of Mr. G. A. McMillan, Department of Scientific and Industrial Research. The Department of Scientific and Industrial Research has appointed Prof. F. H. Garner (Birmingham) and Mr. C. S. Tanner (Imperial Chemical Industries, Ltd.) as 'visitors'.

The Water Research Association commenced with an income of £11,000. This rose to more than £20,000, but the research needs of the water industry are, with the increasing demands for water, so pressing that the water undertakings have decided to double their subscription to the Research Association and the Department of Scientific and Industrial Research has offered grant-in-aid up to £27,000. With an anticipated income of £72,000 the Association is moving its laboratories from Surrey to a site on the Thames in Buckinghamshire, where with an increased staff it can augment its present programme of work on the coagulation process of water purification, leak detection and plastic pipes, by the addition of filtration studies, dewatering of sludge, biological and chemical analytical methods and algal growth control. It will also pursue some of the hydrological interests of the water industry.

Science Information News

The National Science Foundation is issuing a new bi-monthly bulletin, *Science Information News* (1, No. 1; February–March 1959. Published bi-monthly. Annual subscription, 1.75 dollars. Single copies, 25 cents. Washington, D.C.: National Science Foundation, 1959. Obtainable from the Superintendent of Documents, U.S. Government Printing Office), to provide a medium for reporting new and improved methods of disseminating scientific information and news of activities in this field. The first issue (February–March 1959) deals with the establishment by the National Science Foundation of its Science Information Service, the appointment of the Science Information Council and the establishment of the Federal Advisory Committee on Scientific Information and of a Research Advisory Service on Information Processing. There are, however, notes on the International Conference on Information Processing to be held in Paris during June 13–23, on the expanded programme planned by the Office of Critical Tables of the National Academy of Sciences–National Research Council, the study by the Columbia University Bureau of Applied Social Research of the flow of scientific information among scientists, and the study by the Case Institute of Technology of scientific communication among chemists. It is hoped that in future issues the bulk of the bulletin will comprise material contributed by readers and outside the Foundation's own activities in this field.

Royal College of Science and Technology of Glasgow

The latest annual report of the Royal College of Science and Technology, Glasgow, records no major building developments in 1957–58, but of a number of alterations the largest was the conversion of St. Paul's Church for the use of the School of Navigation. This building has yielded accommodation of a high standard and the rooms previously occupied by the school have been taken over by the Department of Natural Philosophy. Full-time students increased from 1,508 in 1956–57 to 1,662, part-time day students from 1,119 to 1,349, while evening students decreased from 2,346 to 2,109. Besides a list of titles of theses presented by 21 members awarded higher degrees by the University of Glasgow, and of those approved for the awards of the postgraduate diploma of the College, the report includes a list of recent publications by members of the College. Further information on research work in progress is given in the Research Report for the Session 1957–58, in which abstracts of most of the publications listed are appended to the summaries of the work in progress in the various Departments.

Jubilee of Queen's University, Belfast

It is just fifty years since, under the Irish Universities Act of 1908, The Queen's College, Belfast, founded in 1845 as part of a government plan for the extension of university education in Ireland, was dissolved and The Queen's University of Belfast was established. The retention of the title 'Queen's' for the new university was intended to show continuity with the foundation of 1845 and to proclaim adherence to the principle of non-sectarian education on which the old Queen's College (and those at Cork and Galway) had been established. This continuity is reflected in the short history of the College and the University, written by Prof. J. C. Beckett, which has been issued as a booklet (Queen's: a Short History of Queen's College, Belfast, and the Queen's University of Belfast. Pp. 10. 6*d*. Belfast: Queen's University, 1959), although the developments of the past fifty years occupy about half the account.

A new building, called the David Keir Building, to accommodate the Departments of Engineering, Chemistry, Zoology and Botany, was opened by the Duke of Edinburgh on May 21. The plans for this extension were described in *Nature* of February 19, 1955, p. 323.

Museum Treatment of Weakened Fabrics

In the issue of the *Museums Journal* of January 1959, Mr. E. R. Beecher describes a method of conserving fragile woven fabric by heat-sealing to prepared nylon net, allowing the intimate overall support provided by adhesion, while preserving the intrinsic textile quality of flexibility, of which some vestige may survive even in the worst example. The nylon net used is described technically as 15 denier 100 per cent bobbin with a count of 42/43 per sq. in. It is sold as 'Nylusion tulle'. Textile fabric thus mounted—full details are to be found in the article quoted—can be examined on both sides. The method is also reversible as a touch of acetone will dissolve the film on the nylon and allow it to be lifted away.

Resin-bonded Coatings of Polytetrafluoroethylene

Thin films of polytetrafluoroethylene on metal and other surfaces are in use because of their properties of low friction, stability at high temperatures, resistance to chemical attack and weak adhesion. Methods of fixing the film on to the base material involve sintering at 400° C., and for this reason films on rubber, plastics, wood and certain metals have not easily been obtained. A dispersion of polytetrafluoroethylene, under the trade name 'Emralon' (Acheson Colloid, Ltd.), has now become available and enables a coating of the polymer to be resin-bonded at low temperatures to a variety of surfaces. The advantage of this coating over the pure polytetrafluoroethylene film is its applicability to heat-sensitive surfaces, although, in addition, stronger adhesion and greater resistance to wear and corrosion are claimed. Of the two grades available, one uses a phenolic resin binder which can be applied by air spray and requires curing for one hour at 150° C.; the second contains a thermoplastic resin binder and needs no heat curing, so that it can be applied to surfaces where baking, even at 150° C., is impracticable.

Geological Society of London: Officers

At the recent annual general meeting of the Geological Society of London the following officers were elected: *President*, Dr. C. J. Stubblefield; *Secretaries*, Mr. J. M. Edmonds and Dr. P. A. Sabine; *Foreign Secretary*, Prof. O. T. Jones; *Treasurer*, Mr. P. Evans; *Vice-Presidents*, Mr. A. J. Butler, Prof. L. Hawkes, Mr. T. C. Nicholas, and Dr. E. I. White.

Institution of Metallurgists: Officers

The following were recently elected to office in the Institution of Metallurgists: *President*, Prof. A. J. Murphy (College of Aeronautics, Cranfield); *President-elect*, W. E. Bardgett (United Steel Companies, Ltd.); *Vice-Presidents*, Dr. W. O. Alexander (Imperial Chemical Industries, Ltd., Metals Division), Prof. J. W. Cuthbertson (University of Nottingham); *Hon. Treasurer*, Dr. E. G. West (Aluminium Development Association).

Royal Society of South Africa: Officers

The following were recently elected to office for 1959 in the Royal Society of South Africa: *President*, A. J. H. Goodwin; *Hon. General Secretary*, W. J. Talbot; *Hon. Treasurer*, Dr. N. Sapeika; *Acting Hon. Editor of Transactions*, Dr. S. Amdurer; *Hon. Librarian*, Dr. J. H. Day. *Other Members of Council*, Dr. S. H. Haughton, Dr. F. G. Holliman, Dr. W. E. Isaac, Dr. R. F. Lawrence, Dr. R. H. Marloth, Dr. S. M. Naudé, Dr. S. H. Skaife, Dr. R. H. Stoy, and Dr. C. L. Wicht.

The Night Sky in June

New moon occurs on June 6d. 11h. 53m. U.T. and full moon on June 20d. 20h. 00m. The following conjunctions with the Moon take place: June 10d. 15h., Venus 7° N.; June 10d. 18h., Mars 6° N.; June 18d. 11h., Jupiter 3° S.; June 21d. 05h., Saturn 4° S. In addition to these conjunctions with the Moon, Venus is in conjunction with Mars on June 14d. 13h., Venus being 0·8° N., and Mercury with Pollux on June 24d. 09m., Mercury being 5·0° S. Mercury will be visible for a short time after sunset during the second half of the month. Venus is an evening star, visible in the south-west after sunset. Conditions are favourable for observation. Venus sets at 23h. 25m., 23h. 05m. and 22h. 30m. on June 1, 15 and 30, respectively; its stellar magnitude is − 3·9. Its distance decreases during the month from 82 to 60 million miles and the visible portion of the apparent disk decreases from 0·607 to 0·455. Mars is in Cancer during the month. It sets at 23h. 30m., 23h. 00m. and 22h. 15m. at the beginning, middle and end of the month, respectively. Its stellar magnitude is about + 1·9; it is rapidly approaching conjunction, and is low in the west after sunset. Jupiter sets at 3h. 20m., 2h. 20m. and 1h. 10m. on June 1, 15 and 30, respectively. It is in retrograde motion in Libra. The stellar magnitude of Jupiter is − 2·0; its distance is approximately 420 million miles. Saturn is in Sagittarius, rising at 21h. 55m., 20h. 55m. and 19h. 40m. at the beginning, middle and end of the month, respectively. Its stellar magnitude is + 0·3 and its distance is approximately 850 million miles. There is one occultation of a star brighter than magnitude 6 visible at Greenwich: June 11d. 21h. 21·2m., 162 B. Vir. (*D*). *D* refers to disappearance. The summer solstice occurs on June 22d. 04h.

Announcements

Dr. Louis Essen, a senior principal scientific officer at the National Physical Laboratory, has been awarded the A. S. Popov's Gold Medal by the Academy of Sciences of the U.S.S.R. This award is for the most distinguished scientific work in the field of radio-engineering performed during the period 1956–58. Dr. Essen has also recently received the first Wolfe Award of the Department of Scientific and Industrial Research (see *Nature*, 183, 930; 1959). The work of scientists from all countries is considered, but this is the first time that it has been awarded to a scientist outside the Soviet Union.

Prof. W. M. Elsasser, professor of theoretical physics at the Scripps Institution of Oceanography and the University of California, La Jolla, California, has been awarded the William Bowie Medal. The Medal is awarded for outstanding contributions to the geophysical sciences.

Dr. Robert T. Parker has been appointed head of the Banbury and Geneva offices of the Aluminium Laboratories, Ltd., in succession to Mr. R. D. Hamer.

Dr. Herman H. Goldstine has been appointed resident manager of the Lamb Estate Research Center of the International Business Machines Corporation, Cortlandt, New York. As resident manager, he will be responsible for IBM research on business theory, psychology, programming, information theory, mathematics, pattern recognition and operational research. Dr. H. J. Greenberg has been appointed manager of the Mathematics Theory Department of the Lamb Estate Research Center.

The Fulmer Research Institute, at Stoke Poges, Bucks., will hold an open day on July 16. Attendance will be by invitation only.

The autumn meeting of the Radiofrequency Spectroscopy Group, which will be held at the Clarendon Laboratory, Oxford, during September 17–18, is to be devoted to the radio-frequency spectroscopy of free atoms and molecules, with sessions on atomic beam and optical methods, microwave gaseous spectroscopy and the measurement of fundamental constants by radio-frequency methods. Registration forms (with requests for accommodation, which must be returned before July 1) can be obtained from Prof. B. Bleaney, Clarendon Laboratory, Oxford.

An International Oceanographic Congress, sponsored by the American Association for the Advancement of Science in co-operation with Unesco and the International Council of Scientific Unions, will be held in the United Nations building, New York, during August 30–September 12. Further information can be obtained from Dr. Mary Sears, Woods Hole Oceanographic Institution, Mass.

The Ninth International Botanical Congress will be held in Montreal, during August 19–29, at the University of Montreal, McGill University and Sir George Williams College, under the auspices of the Canadian Department of Agriculture. Dr. W. P. Thompson, president of the University of Saskatchewan, has been elected president of the Congress. The programme will be conducted in sixteen sections and will deal with all aspects of botany in its broadest sense, including plant production, breeding, and protection and their implications for agriculture and forestry. Inquiries should be directed to the secretary general, Dr. C. Frankton, Ninth International Botanical Congress, Science Service Building, Ottawa, Ontario.

ANTARCTIC METEOROLOGY

A SYMPOSIUM on "Antarctic Meteorology" was held in Melbourne during February 18–25. It was attended by meteorologists from eleven countries. About half the participants had been in the Antarctic during the preceding year, and this gave the discussion an added interest and a feeling of immediacy. The preparatory work carried out at the Commonwealth Bureau of Meteorology, and the smooth organization under the chairmanship of W. J. Gibbs, as well as the friendly hospitality, contributed to the success of the meeting.

The following topics were discussed in seven successive sessions, each with its own session chairman: (1) local effects (K. Langlo); (2) synoptic analysis and forecasting (J. Van Mieghem); (3) synoptic influences on lower latitudes (J. F. Gabites); (4) circulation studies (W. J. Gibbs); (5) snow and ice characteristics (G. de Q. Robin); (6) heat and mass exchanges (C. H. B. Priestly); (7) climatological aspects (J. J. Taljaard).

In practice, certain themes went through the whole symposium and were discussed repeatedly in different sessions from different points of view. One of these was the katabatic wind, which is one of the most spectacular features of antarctic meteorology. Descriptions of local katabatic winds at Mawson, Wilkes, and over the ice cap were given by P. J. R. Shaw, J. Zimmerman and K. B. Mather. The abrupt onset of katabatic winds at D'Urville and other coastal places may be accompanied by an advancing wall of drifting snow; this so-called Loewe's phenomenon was described by B. Valtat. B. L. Dzerdzeevski discussed the effect of the local topography and of the general synoptic situation upon the genesis of the katabatic winds and G. M. Tauber showed that their intensity depends on cyclonic activity over the oceans near the coast. Tauber also discussed the annual and diurnal variation of these winds in coastal regions and suggested that the diurnal variations must cause the turbulent vertical transport of heat to have a maximum during night time. R. H. Clarke described model studies carried out with the aid of a cold dome in a rotating dish pan. One of the most interesting papers on katabatic winds was presented by F. K. Ball. It gave the theory of airflow under an inversion along a rough, inclined surface. The theory indicates that katabatic stream lines should form an angle of about 40° with the fall line. Along the coast, this causes relatively strong winds at the eastern side of a headland or on the western shores of a bay. It also causes particularly strong katabatic winds in the south-west quadrant of off-shore depressions. These theoretical predictions are in good agreement with observations and with Tauber's investigations.

Another subject which came up repeatedly in different sessions was the intense heating which occurs in the antarctic stratosphere in spring. The antarctic stratosphere at 20 km. cools gradually by about 60° C. from February to September. With the return of the Sun the stratosphere warms up again; a large part of the warming (65 per cent) is accomplished within a few weeks towards the end of October or early November. K. Hanson presented a case-study of this explosive stratospheric warming over the Antarctic in October 1958. Conditions in 1957 were described by E. Flowers. Comparing arctic and antarctic conditions, H. Wexler (whose paper was presented in his absence by M. J. Rubin) directed attention to the fact that the heating in the Arctic is slightly less pronounced and that it occurs before the return of the Sun in February. In the Antarctic it occurs well after the return of the Sun. Wexler explained the spectacular warming of the polar stratosphere by a change in the circulation patterns which leads to large-scale subsidence. This view is not easily reconciled with investigations into advection across the antarctic boundary by M. J. Rubin, whose studies showed no evidence of systematic divergence from the Antarctic in the troposphere and lower stratosphere during summer. Rubin, however, did not study specifically the period in which the stratospheric heating occurs. E. B. Kraus showed that the heating cannot be due to direct absorption of solar radiation. He suggested that it may be caused by advection of moist warm air in the troposphere which results in a change in the long wave-lengths radiation balance above. This view would be in agreement with Hanson's case-study, which indicates a warming of the lower polar troposphere a few days before the rapid stratosphere heating occurred.

As regards equipment, much interest centred on automatic weather recording systems. American automatically reporting weather stations which did not require manual erection and could be dropped from the air were described by W. S. Lanterman. An automatic non-recording climatological station which had been left by the Japanese expedition at Syowa Bay during winter 1958 was discussed by Y. Morita and N. Murakoshi. Traps to measure the lateral transport of snow by drifting were tested by M. Mellor at Mawson. From an analysis of these drift measurements it was deduced by Mellor and U. Radok that the lateral transport of snow was even larger than had been anticipated hitherto. To gain maximum height for upper air measurements a special balloon valve had been developed at the University of Melbourne. V. D. Hopper stated that balloons equipped with this valve reached generally a height of 35 km. with a maximum of 43·5 km. The result of ozone observations at Halley Bay were described by J. MacDowall.

J. F. Gabites gave a lucid account of the antarctic heat balance. He found the flow of heat across 55° S. to be of the same order or only a little higher than the flow across 55° N. in spite of the greater meridional temperature difference. This could be explained by the smaller effect of friction over the ocean-covered southern hemisphere, which allows the establishment of greater vertical wind gradients and therefore greater horizontal temperature and density gradients.

Circulation in the upper air was the subject of a paper by W. B. Moreland and was also discussed in several other contributions. A stratospheric jet is a well-marked feature of the winter circulation. As in the Arctic, this polar night jet appears to be synoptically separated from the systems of the troposphere. There are, however, significant differences between the two polar regions. In the Arctic the jet core is commonly found at the 25-mb. level; in the

south it occurs at a lower elevation. The jet stream persists there also later into the polar day, as may be expected from the later heating of the antarctic stratosphere. It has not been possible so far to investigate synoptically the effect of the final collapse in spring of the polar night jet upon the circulation pattern in lower latitudes.

The scarcity of observations from the Southern Ocean area inevitably affected general circulation studies and the discussion of the interaction between the Antarctic and lower latitudes. In presenting his paper on antarctic synoptic analyses, W. J. Gibbs suggested that the presence and the main features of cyclones and anticyclones can be diagnosed by the analyst though there was still considerable doubt about the reality of frontal analyses. Synoptic studies of cold outbreaks and surges had been prepared by a number of Australian authors (A. K. Hannay, R. Falconer and H. M. Treloar, F. A. Berson and U. Radok). Circulation studies based on synoptic material were presented by J. Langford, I. S. Kerr, H. van Loon and S. Karelski.

Synoptic conditions over the antarctic ice cap itself are now reasonably well known for the first time. The communication system required to achieve this end was described by T. I. Gray, jun. It appears that cyclones do occasionally penetrate deep into the Antarctic and that fronts move sometimes right across the South Pole, though this occurs not as often as is probably the case in the north. In general, high-latitude cyclones seem to move sluggishly along the coast.

The happy international co-operation established between meteorologists in the Antarctic is perhaps symbolized by a paper submitted by P. D. Astapenko (U.S.S.R.) which was based on a joint investigation by him, J. Alt (France) and M. Ropar (United States). It is hoped that this co-operation will be continued in the International Antarctic Analysis Centre which is now being set up under the ægis of the Australian Bureau of Meteorology at Melbourne and that the work carried out there will lead to a better understanding of meteorological problems as a whole.

E. B. Kraus

HYDROGEN LIQUEFACTION

THE use of liquid hydrogen, first produced and collected by Dewar at the Royal Institution in 1898, has, until recently, been largely confined to the laboratory. With a normal boiling point of 20·4° K. it provides a convenient means of controlling the temperature for experiments in the range 14°–90° K. and it is used as a precooling agent in the liquefaction of helium.

On March 12, a one-day symposium on "Hydrogen Liquefaction and Associated Techniques", organized by the Low Temperature Group of the Physical Society, was held at the Institute of Physics in London, and it was evident from the attendance at the meeting, as well as the topics discussed, that interest in the liquefaction of hydrogen on an industrial scale is developing in Great Britain.

The morning session, under the chairmanship of Dr. P. H. Sykes, was opened by Prof. G. O. Jones (Queen Mary College, London), who spoke of the applications of liquid hydrogen in pure research. He pointed out that it is necessary to go well below temperatures obtainable with liquid hydrogen in order to study the interesting features of many phenomena. Nevertheless, liquid hydrogen is of great technical assistance in many experiments. In optical spectroscopy, for example, the reduction in thermal motion of atoms at low temperatures results in a decreased Doppler broadening of spectral lines. The use of liquid hydrogen in bubble chambers, where it may form both a target and a detector for nuclear reactions, has also proved of great value.

In certain experiments, however, there is little point in working at temperatures below 20° K. For example, the thermal expansion coefficients of most substances and variations in such properties of the solidified inert gases as the density, compressibility and refractive index become so small below 20° K. that they are difficult to measure with precision. Such experiments may very conveniently be performed in miniature hydrogen liquefiers with about 100 ml. of liquid and in which liquefaction commences some 15 min. after starting at room temperature.

The design and construction of small-scale liquefiers were discussed by Dr. D. H. Parkinson (Radar Research Establishment, Malvern), who emphasized the ease with which liquid hydrogen may be produced in the laboratory with relatively simple and inexpensive apparatus. High-pressure hydrogen, usually from a cylinder, is precooled with liquid nitrogen and then expanded at a valve into a reservoir. A proportion of the gas liquefies and the remainder is used to cool the incoming high-pressure gas by means of a heat exchanger between the liquid hydrogen and nitrogen stages. The yield of liquid depends rather critically on the pressure and precooling temperature; for example, a standard 120 cu. ft. capacity cylinder exhausted from 120 to 50 atmospheres would yield 0·36 litre of liquid if the gas were precooled to 77° K. and 0·55 litre with a precooling temperature of 65° K. Impurities, usually amounting to about 0·1 per cent in commercial hydrogen, must be removed from the gas or they will solidify and block the liquefier tubes, and 150 gm. of activated charcoal cooled in liquid nitrogen was recommended as an adequate absorber for each cylinder of gas.

The use of liquid hydrogen as a source of 'cold' has already been mentioned, and an application to the production of a beam of low-energy neutrons was described by Dr. F. J. Webb (Atomic Energy Research Establishment, Harwell). Neutrons from a reactor with, say, a heavy-water moderator at a temperature of 50° C. are thermal neutrons, that is, slow neutrons with energies of the same order as the thermal energies of atoms in a solid. They are spread over a range of values with the greatest number possessing energies corresponding to a de Broglie wave-length of about 1 A. In certain diffraction experiments, however, it is necessary to employ neutrons with wave-lengths greater than 4 A., the number of which is relatively small compared with those of 1 A. wave-length. Since the thermal energy is proportional to the absolute temperature, the kinetic energies of the neutrons may be reduced, and their wave-lengths correspondingly increased, by using liquid hydrogen as a moderator. In the *Dido* reactor at Harwell, a small hydrogen liquefier feeds a 3-in. diameter container placed in the neutron beam and the liquid hydrogen moderator results in a gain of the order of

25 in the flux of neutrons of the required wavelength.

Another application of liquid hydrogen to pure research was described by Mr. C. van Zyl (University College, London), who spoke of its use as a scattering target for high-energy particles. Interactions between two particles such as the proton and the neutron are being investigated as an approach to an understanding of the many-body problem. By employing three targets, one of liquid hydrogen, one of liquid deuterium, and a blank target to eliminate background scattering, it is possible to study *n–p*, *p–n*, *n–n* and *p–p* reactions in the same apparatus.

One of the difficulties encountered in building apparatus for low-temperature work is that of providing vacuum-tight windows for particles or radiation without causing appreciable absorption or scattering. Mr. van Zyl described windows for the target chambers made of thin (0·002 in.) 'Terylene' film which will contain liquid hydrogen when the chambers are surrounded by a high vacuum.

As every schoolboy knows, the considerable size of a space rocket is largely accounted for by the quantity of fuel required to deliver a given load, and the search for more exotic fuels proceeds apace. The ultimate performance of a ballistic vehicle depends upon its 'burn-out' velocity, and this is inversely proportional to the square root of the molecular weight of the fuel. Thus, the best rocket fuels have low molecular weights and are generally rich in hydrogen. From this aspect, liquid hydrogen is obviously a desirable choice, and in the afternoon session, under the chairmanship of Dr. M. Ruhemann, the possibilities were discussed by Mr. A. V. Cleaver (Rolls Royce, Ltd.).

It is estimated that combustion of hydrogen–oxygen mixtures would increase the thrust by some 40 or 50 per cent over that obtainable with conventional kerosene and liquid oxygen fuels. Against this must be set the complications of accommodating a liquid of low boiling point and low density which would tend to reduce the quantity of propellant carried by the rocket. However, recent improvements in the construction of fuel tanks, so that they contribute to the structural rigidity of the rocket and reduce its unladen weight, would seem to make the use of liquid hydrogen more feasible. Although no work is being conducted in Britain, encouraging results have been achieved in the United States with small rocket motors using liquid hydrogen and liquid oxygen.

Clearly, if liquid hydrogen were successfully employed as a fuel, the demand would exceed present production capacity very considerably. With this possibility in mind, Mr. W. L. Seddon (Petrocarbon Developments, Ltd.) presented a design study of a plant capable of producing 2 tons (about 30,000 litres) of liquid hydrogen per hour. This may be compared with one of the few large plants in existence, that at Boulder, Colorado, with a potential capacity of 600 litres per hour.

Hydrogen gas would be derived from oil refinery light hydrocarbon products, and since it is difficult to predict the future demand for liquid hydrogen the economics of the scheme were based on the production of heavy water by distillation. Liquefaction would take place by Joule–Thomson expansion of compressed hydrogen, but one difficulty would be the removal of impurity, $\frac{1}{2}$ per cent of nitrogen present in the feed gas resulting in the deposition of 300 lb. of solid per hour. It was therefore proposed to precool the feed gas to about 35° K. with the aid of expansion turbines and expand the gas from a pressure of about 16 atmospheres. Under these conditions, the efficiency of the Joule–Thomson process remains fairly high and a considerable proportion of impurity is carried through the expansion valve and deposited in the liquid reservoir, where it may easily be removed.

The capital cost of such a plant is in the region of £6,000,000, and the estimated price of the product, allowing for the sale of heavy water as a by-product, is £200 per ton. Liquid hydrogen produced on this scale would therefore work out at a cost of about 1*s.* a gallon, comparing surprisingly favourably with hydrocarbon fuels at, say, 4*s.* 8*d.* a gallon.

On a somewhat smaller scale, a paper by Dr. B. Shaw and Mr. W. H. Denton dealt with the problem of coping with a varying demand at the Atomic Energy Research Establishment, Harwell, by storing liquid hydrogen in 50-litre Dewar vessels. Hydrogen consists of molecules in two different rotational states, the equilibrium concentration being 75 per cent *ortho*-hydrogen and 25 per cent *para*-hydrogen, but the liquid is slowly converted to the *para* form with evolution of heat. In storage, therefore, liquid is lost by *ortho–para* conversion and it is usually more desirable to induce the conversion before liquefaction by passing the high-pressure gas over a catalyst such as ferric oxide or activated charcoal at low temperatures. Dr. Shaw presented figures showing that, for short-term storage, greater overall economy is gained by carrying out partial conversion at 80° K. than by converting completely to the para form at 20° K. The total loss from a 50-litre Dewar vessel with liquid nitrogen shielding may be as low as 0·18 litre per day.

For storage on a large scale, however, a vacuum-insulated vessel employing a liquid nitrogen radiation shield would be unduly large and complicated and the feasibility of using thermal insulation similar to that now employed in liquid nitrogen and oxygen storage vessels was discussed in a paper by Mr. K. C. Smith and Dr. J. B. Gardner (British Oxygen Research and Development). In these vessels, the liquid is surrounded by a space evacuated to a pressure of 10^{-1} mm. mercury and packed with an insulating powder. For liquid hydrogen, the loss from such a vessel would chiefly be due to radiation, and Mr. Smith described measurements of the overall thermal conductivity resulting from the addition of reflecting particles, such as aluminium powder, to the powders commonly in use. They show a significant decrease in thermal conductivity and indicate that the thickness of insulation need not be inconveniently great in, for example, a vessel of 10 ft. in diameter. Similar considerations are being applied to the design of transfer lines for large quantities of liquid.

The question of safety must be given prime consideration when dealing with liquid hydrogen, and some general remarks were made by Dr. A. J. Croft (Clarendon Laboratory, Oxford), who stressed the importance of making provision for possible human errors in operating liquefiers. Many of the precautions are common to any equipment containing low boiling-point liquids or gases at high pressure, as, for example, the fitting of relief valves to prevent the pressure rising above a safe value. One must also adopt the precautions normal when dealing with a highly inflammable liquid or explosive vapour, such as adequate ventilation of buildings. The con-

sequences of a mishap should be minimized by partly enclosing liquefying plant by blast walls and by operating it remotely. This, of course, is not always feasible, particularly with small-scale laboratory installations.

Happily, there have been few serious accidents attributable to the use of liquid hydrogen, and reasonable and sensible safety precautions would appear to suffice. Judging by the papers and discussions presented at this symposium, it is conceivable that, in the future, liquid hydrogen will be produced and handled on a scale and with a facility comparable to that encountered to-day with liquid oxygen.

D. H. Bowen

PARASITOLOGY

THE first meeting outside London of the Parasitology Group of the Institute of Biology was held in the Zoology Department, University of Edinburgh, during March 19–21. It was the longest session so far held and the range of topics discussed was limited only by the general heading of "Parasitology".

Twenty-five papers were read, covering subjects as varied as the use of antigen–antibody reactions to differentiate species and strains of *Entamoeba* (V. Zaman), and the morphology, function and taxonomic status of the adhesive apparatus of Amphibdellatid parasites of electric rays (J. Llewellyn).

Some of the speakers were concerned with the mechanisms which trigger the changes in parasite life-histories. E. W. McConnachie reviewed the possibility that the onset of sexual reproduction of some parasites is related to hormonal changes in the host, and outlined her preliminary experiments with *Opalina* in frogs which tentatively support this view. Assuming a direct effect of the host's hormones on the parasite, one would have to postulate the presence of such hormones at the site of parasitism, which, for *Opalina*, is the lumen of the frog's rectum. Coccidian Protozoa would seem to be in a more favourable position to be influenced by systemic changes in the host since they develop in the epithelial tissues of the gut, but S. F. M. Davies showed that no mechanism of this kind was needed by *Eimeria necatrix* in chicks, the sexual phase of development being initiated by second-generation merozoites irrespective of the physiological state of the host.

The evolution of a temperature trigger mechanism in the life-cycle of certain tapeworms was argued by C. A. Hopkins on the basis of ecological studies on *Proteocephalus filicollis* in sticklebacks. He showed that in the north of Britain very few of the immature stages of these worms complete their development, even though they become successfully established in the fish host. Comparison with the state of affairs in the south suggested that low temperature can arrest development of such a species, limited, as it is, to cold-blooded hosts. An elegant solution to the parasite's problem would be the inclusion in the life-cycle of an 'incubator' in the form of a warm-blooded host. Hopkins suggested that this may have happened in the case of three-host pseudophyllidean tapeworms, since a rise in temperature is almost sufficient on its own to mature the plerocercoids of some of these parasites.

Mechanisms which enable free-living stages to locate their hosts were considered in two papers, one by C. A. Wright, the other by C. Ellenby and A. B. Gilbert. The former put forward evidence in favour of a response by trematode miracidia to substances exuded by their molluscan hosts. Ellenby and Gilbert presented a progress report on work with the hatching factor of potato root eelworm and showed that their results were still consistent with the view that an unsaturated lactone ring is diagnostic of the molecule of the active constituent of potato root diffusate.

Problems more comprehensive than trigger mechanisms were considered by E. J. Bell, C. A. Hopkins and M. R. Wyllie. These workers described their joint efforts to bridge the gap between the analytical and synthetic aspects of the *in vitro* culture of strigeid metacercariae to the sexually mature adult fluke. Media synthesized from chemically defined and simple constituents were very limited in their ability to promote growth and had to be assessed by sensitive criteria, such as cell-division, rather than by an increase in size of the organism or its parts. On the other hand, some of the unknowns of complex media which do support considerable growth could now be replaced by known substances, but the gap between the two approaches was still formidable.

Experiments which illustrate the differences that may exist between the metabolism of host and parasite were described by L. P. Joyner. Both sulphadimidine and pyrimethamine on their own showed therapeutic effect when administered to chicks infected with *Eimeria tenella*, but the two administered together gave more than additive effect. This is consistent with the theory that they antagonize different links in the same metabolic chain of the parasite, namely, the *p*-aminobenzoic acid – folic – folinic acid sequence. The drugs are also toxic to the chick, but when they were administered, together with the metabolites they are presumed to antagonize, differential reversal of their effects on host and parasite occurred.

Investigations into mechanisms concerning resistance of hosts to their parasites were reported by a number of speakers. F. W. Jennings presented the findings of a team of workers on immunity to *Dictyocaulus* infection in cattle. He showed that a double 'vaccination' of the host with irradiated infective larvæ protected it against further challenges of normal larvæ. L. Bailey dealt with the resistance of honeybees to epidemics of a range of parasites, and described how, without the aid of acquired immunity, the behaviour of the colony as a whole constituted a 'group immunity' reaction, and was successful in combating the disease as long as the community could develop normally. This was in part analogous to the dynamics of *Sarcoptes scabiei* infections in man, considered in two papers by K. Mellanby and W. N. Boog Watson, though in this case acquired immunity was an additional factor. The breakdown of resistance to infection in 'resistant' strains of potato by certain biotypes of potato root eelworm, and the investigation of its genetic basis in the nematode, was discussed by F. G. W. Jones. The evidence suggested a single locus for the effect, the double recessive conferring resistance-breaking, though the fact that some of the heterozygotes were resistance-breakers was against this simple interpretation.

The advantages of such a wide field at a conference of this kind were illustrated by the implications of the use of resistant species or strains to reduce the number of infective stages available to others that are susceptible. This is known to be valuable in the case of potato root eelworm, where resistant strains of the plant nevertheless stimulate hatching of the cysts, and was suggested by H. D. Crofton and P. G. Yonge as a mechanism in the control of *Diphyllobothrium* infections in trout when a normal proportion of coarse fish is present to reduce the level of infective material.

A plea to parasitologists not to lose sight of the economic aspect in the aims they set themselves when investigating the parasitic diseases of domestic animals was put forward by M. Christie. However, in the case of infections in man the parasitologist has a duty to assist in divesting him of his parasites. Contributions with this goal in mind included a mathematical analysis by W. E. Kershaw of the effects of parasitic infections on the mortality of insect vectors. These effects differed with the parasite and the vector, but the ability to support the parasite decreased with increasing age of the insect host, so that the vector may be present and yet incapable of transmitting the disease. Studies reported by A. I. Wright revealed an understandable, but nevertheless alarming, lack of knowledge of the etiology of hydatid disease on the part of some keepers of foxhounds in England, and showed that dogs fed on raw meat and offal readily become infected with *Echinococcus granulosus*.

No meeting of this kind would be complete without the description of a hitherto unknown animal. This was provided by J. Mahon, who presented studies on a new tapeworm, together with her reasons for placing it in the family Dilepididae.

P. A. G. Wilson

HORTICULTURAL AND FRUIT RESEARCH

THE agricultural side of the work of the Agricultural and Horticultural Research Station at Long Ashton has been concerned mainly with fruit farming, but the retiring director, Prof. T. Wallace, had earlier described and photographed in colour the symptoms of numerous mineral deficiencies on a wide variety of crops. This raised so many questions that a research unit on plant nutrition was formed and is now firmly established at the Station under the ægis of the Agricultural Research Council. The annual report for 1957*, which was recently published, includes results of current work by this Unit on molybdenum deficiency, which has complex relations with nitrogen sources and with phosphate. A new type of symptom has indeed been produced when ammonium sulphate was used as a nitrogen source in place of nitrate. Other work of the Unit is outlined in the research summary of the report; much of it is obviously of a preparatory nature, but the results should ultimately provide knowledge upon an altogether new plane of detail for studies in plant nutrition.

In the main part of the report, C. Bould reports that two synthetic soil conditioners, HPAN (sodium salt, hydrolysed polyacrylonitrile) and VAMA (calcium salt, vinyl acetate maleic acid co-polymer), do indeed improve soil structure, but bring no increase in yield of strawberries. L. C. Luckwill and A. I. Campbell have obtained successful control of bindweed growing among black currant bushes by spraying 300 gal. per acre of 0·1 per cent potassium salt of 2 : 4 : 5-trichlorophenoxybutyric acid (2 : 4 : 5-TB). Long Ashton has undertaken the propagation of a virus-tested clone of loganberries, and E. W. Hobbis and A. I. Campbell have worked out a technique of leaf-bud propagation for its more rapid accomplishment. Barbara A. Rake reports that gooseberry cuttings taken from fruiting bushes root more easily than those taken from younger plants, and A. I. Campbell finds that crab *C* rootstocks are necessary to provide sufficient stem for cider-apple standards. The stem building stock Bulmer's Norman could also, however, be double worked satisfactorily upon a variety of rootstocks. D. Wilson finds that the prolific new pear variety, Bristol Cross, introduced by the Station in 1933, is almost completely male sterile, and should therefore be grown only with a pollinating variety, for example, Conference.

Plant pathology occupies a large part of the report. L. C. Luckwill has obtained evidence that 'chat fruit' disease of the apple Lord Lambourne is transmissible by graft and of virus origin. Spores of the fungi causing apple and pear scab diseases require a period of wetting before they will germinate, the so-called 'Mills periods'. Most of the work on these periods, which have forecast value, was done with ascospores of *Venturia inaequalis* (apple scab), but D. Wheatley shows that conidia of this fungus gave a higher percentage germination and grew more rapidly than ascospores. Conidia of *V. pirina* grew even faster than conidia of *V. inaequalis*, and these facts would necessitate a revaluation of Mills' forecast periods to meet particular situations, according to whether ascospore or conidial infection was involved. Several losses of stored apples have occurred in recent years; they are caused by the fungi *Gloeosporium perennans* and *G. album*, which infect the fruit while still on the tree. R. O. Sharples finds that the fungi complete their life-cycles as weak parasites on apple shoots, that fruit infection is incidental, and that spore production can be reduced by sprays or wound paints containing phenyl mercury chloride. R. T. Burchill shows that apple mildew, *Podosphaera leucotricha*, infects the buds via the petioles, a bud remaining susceptible to attack for about a month after emergence.

R. J. W. Byrde and Nora N. Waugh report laboratory tests of eleven new potential fungicidal materials. Three of them—*n*-dodecyl-guanidine acetate, 2 : 4-dichloro-6-(*o*-chloroanilino)-*s*-triazine, and *o*-hydroxydiphenyl—were selected for field tests. Bordeaux mixture is usually made by adding concentrated copper sulphate solution to diluted lime suspension, but E. Somers shows that better performance can be expected if the lime is concentrated and the copper sulphate dilute. R. J. W. Byrde, G. M. Clarke and C. W. Harper have again found in 1957 that captan used as a post-blossom spray on apples increased the incidence of mildew. J. T. Martin and J. A. Pickard

* University of Bristol. The Annual Report of the Agricultural and Horticultural Research Station (The National Fruit and Cider Institute), Long Ashton, Bristol, 1957, with Index 1953–57. Pp. 182 + 10 plates. (Long Ashton, Bristol: Agricultural and Horticultural Research Station, 1958.) 15s.

have studied the retention of mercury salts by apple fruits after seven sprays with 0·003 per cent mercury as phenyl mercuric nitrate. A fruit weighing 200 gm. had about 10 μgm. of mercury: 2 μgm. in the peel, 8 μgm. in the flesh and less than 1 μgm. in the core. T. E. Cobbold describes a compressed air nozzle for small-volume spraying, and has also assessed the field performance of the power-knapsack type of mist sprayer. Little is known about the chemical composition of the cuticle in different plants, but J. T. Martin, R. F. Batt and Margaret F. Roberts show that there are considerable variations in the overall amounts of cuticle and in the proportions of wax and cutin between plants. As the cuticle is the initial receptor of sprays, a knowledge of its constitution should be important in practice.

There are also papers on domestic food preservation, on fruit juices, and on the conversion and utilization of the Station's plantation of cricket bat willows. Research workers and advisory officers will find a most useful list of papers published by members of the Station's staff in 1957, and there is a further index of papers published in the Long Ashton annual reports during 1953–57.

JOHN GRAINGER

INSECT RESISTANCE TO INSECTICIDES

THE discovery by Müller of the insecticidal properties of DDT in 1939 was followed by the introduction of a whole range of similarly powerful synthetic insecticides in the years immediately after the Second World War. These included other chlorohydrocarbons like dieldrin, the gamma isomer of benzene hexachloride, and a number of organophosphorus compounds. Their use marked a new era in the control of arthropod pests and the development of what is now an important branch of the chemical industry. The spectacular control of the malarial mosquito, for example, brought the global eradication of the disease within the realm of probability.

The development of insect resistance to some of these insecticides, however, now threatens to undermine much of the progress achieved and the hopes of some large-scale public health programmes in tropical latitudes especially. Resistance may attain such levels as to involve virtual immunity of the insect to an insecticide and the failure of effective control in the field. Yet remarkably little is known about the mechanisms of resistance and in no case has a satisfactory counter-measure been developed except to change to one of the dangerously few alternative insecticides. A major problem has been to engage the interest of suitably equipped and experienced workers and to facilitate the exchange of information and insect material between research workers and the often far-distant field entomologist or sanitarian. A good deal has recently been achieved by a small dedicated group of the World Health Organization in Geneva due in no small part to the tireless efforts of Prof. A. W. A. Brown. His recent monograph on "Insecticide Resistance in Arthropods"* is one result of a two-year term-of-office with the Organization and reflects an authority and erudition made possible only by a combination of the uniqueness of the author's position and his personal industry.

Four long chapters deal with the genetic nature and development of resistance, resistance in species which are vectors to man, what is known of the physiological and biochemical mechanisms of resistance, and resistance in species which are not vectors.

The first chapter describes the historical development of resistance in vector species, the public health implications, the genetic nature of the inheritance of resistance, and the important problem of detecting significant levels of resistance in field populations. The second chapter deals *seriatim* with case histories of the vectors such as body lice, and mosquitoes transmitting malaria and yellow fever. The third chapter deals mainly with the housefly, since much the larger part of systematic research has been conducted with this insect. The final chapter, like the second, deals with case histories of the field development of resistance in fruit flies, bed bugs and cockroaches.

The monograph will interest the geneticist as well as entomologist and biochemist. There is abundant evidence that resistance is a pre-adaptive phenomenon and under genetic control, so that the development of resistance to an insecticide is due to selective breeding from naturally resistant phenotypes. The easy culture of highly resistant strains in the laboratory has probably provided an unprecedented opportunity for studying the biochemistry and genetics of evolution.

Prof. Brown has wisely refrained from speculating about the future of the resistance problem and the possible development of countermeasures. These may indeed involve entirely new concepts in the field of insect control. On the other hand, there are grounds for believing that effective counter-measures can be developed on the basis of the continued use of insecticides: for example, the exploitation of enzymes which appear to be present in far higher concentrations in the tissues of a resistant insect than in a susceptible one, or the alternate use of insecticides negatively correlated in their respective toxicities towards susceptible and resistant strains.

Some readers may be disappointed to find relatively little reference to resistance of agricultural pests and its implications. For example, there is no mention of the serious levels of resistance to the chlorinated hydrocarbons, reported in 1957 to have developed in the cotton boll-weevil infesting the larger cotton-growing area of Louisiana. While such an omission is not surprising in a publication of the World Health Organization, it might have been usefully indicated in the title or in a sub-title of the monograph. Prof. Brown has, perhaps, drawn a little heavily on pre-publication material some of which has shown signs of being a little premature. These blemishes notwithstanding, the monograph is a timely and invaluable contribution to an appreciation of this big problem. It is recommended as salutary reading to all interested in the chemical control of arthropod pests and as an essential text to all concerned with the problem of resistance itself.

F. P. W. WINTERINGHAM

* World Health Organization. Monograph Series No. 38: Insecticide Resistance in Arthropods. By Dr. A. W. A. Brown. Pp. 240. (Geneva: World Health Organization; London: H.M. Stationery Office, 1958.) 15 Swiss francs; 25*s.*; 5 dollars.

METABOLISM OF MYLERAN (1 : 4-DIMETHANESULPHONYLOXYBUTANE)

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EARLIER publications[1,2] on the mode of action of biologically active alkylating agents described the presence of derivatives of S-alkylated cysteines in the urine of rats given alkyl alkanesulphonates by intraperitoneal injection. Thus, for example, 1-^{14}C-ethyl methanesulphonate (I) was shown to be excreted as N-acetyl-S-1-^{14}C-ethylcysteine (II) and other derivatives of S-1-^{14}C-ethylcysteine.

$$\overset{*}{C}H_3.SO_2.O.CH_2.CH_3 \longrightarrow \overset{*}{C}H_3CH_2.S.CH_2.CH(NH.COCH_3).COOH$$

I II

This work was regarded as a prelude and guide to the investigation, now reported, of the *in vivo* reactions of 1 : 4-dimethanesulphonyloxybutane (myleran) (III) ; $n = 4$; a cytotoxic alkylating agent used clinically in the treatment of chronic granulocytic leukæmia[3]. These studies also provide a comparison between the *in vivo* reactions of two structurally related compounds, one monofunctional and the other bifunctional. Myleran is far more toxic to the rat than ethyl methanesulphonate and, unlike the latter, exerts a profound effect on the bone marrow, which results in a drop in the number of circulating neutrophils[4].

$$CH_3.SO_2.O\,(CH_2)_n.O.SO_2.CH_3$$

III

By analogy with ethyl methanesulphonate it was anticipated that myleran would react *in vivo* with the thiol group of cysteine or cysteine-containing compounds (peptides or proteins), resulting in the urinary excretion of metabolites of S-dicysteinylbutane (IV). However, evidence for such a reaction product was not obtained, while it was established that injected S-dicysteinylbutane was excreted in the free and conjugated forms. Furthermore, S-(4-hydroxybutyl)cysteine (V), which would be formed if one functional group of myleran reacted with cysteine while the other was hydrolysed, was also shown to be excreted as conjugates of sulphur compounds which were detectable on paper chromatograms. These compounds were not present on similarly prepared chromatograms of urine from rats which had received injections of myleran.

$$COOH.CH(NH_2).CH_2.S.(CH_2)_4S\,CH_2.CH(NH_2).COOH$$

IV

$$OH.(CH_2)_4.S.CH_2CH(NH_2).COOH$$

V

After injection of 2 : 3-^{14}C-myleran (prepared by Dr. P. Brookes) into a male Wistar rat, only 4 per cent of the radioactivity was exhaled as carbon-14 dioxide during 24 hr. On the other hand, 2 : 3-^{14}C-1 : 4-butanediol, the hydrolysis product of myleran, was shown to be converted almost exclusively to carbon dioxide labelled with carbon-14 *in vivo*, indicating that myleran is not metabolized via this route. It will be recalled that conversion to carbon dioxide was one of the major routes of metabolism of ethyl methanesulphonate.

Autoradiographs of paper chromatograms of the urine collected during 24 hr. after injection of myleran revealed the presence of one major metabolite, which was stable to treatment with hot mineral acid and failed to give any positive test (on paper chromatograms) with a number of reagents such as ninhydrin or platinic iodide. The necessarily minute doses of myleran which were given to rats precluded isolation of adequate quantities of the metabolite for characterization.

In view of these apparent differences between the metabolism of ethyl methanesulphonate and myleran, the reaction between myleran and cysteine *in vitro* was studied in greater detail. Apart from the formation of some S-dicysteinylbutane and S-(4-hydroxybutyl)cysteine the major product of the reaction under a variety of conditions was the S-β-alanyltetrahydrothiophenium cation (VI).

$$[CH_2(CH_2)CH_2S^+CH_2.CH(NH_2).COOH]$$

VI

The ion was characterized as its picrate and was unambiguously synthesized by heating S-(4-hydroxybutyl)cysteine with concentrated hydrochloric acid.

The urine obtained from a rat which had been injected with this sulphonium salt (VI) (prepared from the labelled myleran) also contained one major metabolite identical with that formed from myleran, as judged by comparing autoradiographs of identically prepared paper chromatograms of the respective urines. The identity of this major metabolite has still to be determined ; but since it is no longer an amino-acid it is clearly a break-down product of the cyclic sulphonium ion (VI), possibly a derivative of tetrahydrothiophene. The sulphonium salt (VI) was shown to be labile in the presence of alkali and was converted to alanine by electrolytic reduction.

These results indicate that the predominant reaction which myleran undergoes in the rat is alkylation of a thiol group, and by analogy with ethyl methanesulphonate probably that of cysteine or cysteine-containing compounds, to give a cyclic sulphonium salt, which is the precursor of the major urinary metabolite.

Certain sulphonium ions have been shown to possess alkylating ability, depending both on the

nature of substituents and the associated anion. However, the fact that S-dicysteinylbutane or S-(4-hydroxybutyl)cysteine is not produced when (VI) is treated with excess cysteine or water, and that it is not metabolized to these compounds *in vivo*, would suggest that this sulphonium ion is not an alkylating agent. This is supported by the fact that it was found to be non-toxic to the rat, and did not produce myleran-like effects on the bone marrow at a dose as high as 1 gm./kgm. body-weight (L. A. Elson, private communication). It seems likely, therefore, that this compound is not responsible for the pharmacological effects of myleran; but the possibility remains that the ion is unable to penetrate the necessary cell boundaries to produce its effects. Those pharmacological properties of myleran, therefore, which result from reaction with thiol groups are likely to be due to reaction with thiol groups of proteins or nucleoproteins leading to sulphonium ion formation. The formation of such ions may offer an explanation for the differences in pharmacological properties which have been observed between myleran and its monofunctional counterpart, ethyl methanesulphonate. While both compounds have been shown to react with thiol groups *in vivo*, the formation of sulphonium ions from myleran may have more far-reaching effects by modifying the structure and function of macromolecules. It has also been found that in the series of *bis*-methanesulphonyloxy esters[4] (III; $n = 2$–10) maximum toxicity and maximum activity against the neutrophils occur when $n = 4$ or 5, distances which would permit a facile cyclization to five- or six-membered rings, respectively[5]. This view is supported by some preliminary *in vitro* chemical studies of the reaction between cysteine and various members of the series (III; $n = 3$, 5 and 6).

Other alkylating agents such as the sulphur and nitrogen mustards could conceivably undergo *in vivo* ring formation in an analogous manner and work is in progress to determine whether this is the case.

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WEAL AND FLARE IN HUMAN SKIN PRODUCED BY HISTAMINE AND OTHER SUBSTANCES

By DR. A. HERXHEIMER and DR. M. SCHACHTER

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ALTHOUGH histamine is the most prominent wealing agent in man, evidence is accumulating which indicates that other naturally occurring substances have a similar action on capillary permeability. Among substances that have been found to have this property in laboratory animals are kallikrein[1,2], kallidin, bradykinin, wasp kinin[3] and 5-hydroxytryptamine[4]. The present experiments demonstrate that wasp kinin, kallidin, bradykinin and kallikrein preparations are effective wealing agents in man on intradermal injection. Also, that the size of flare and the size of weal produced by these substances do not run parallel. For example, kallikrein, which increased capillary permeability, produced no flare, whereas 5-hydroxytryptamine, which was an effective flaring agent, failed to cause wealing or other evidence of increased capillary permeability. Until pure substances are available the possibility exists that the permeability-enhancing agents in our preparations are not identical with the smooth muscle contracting (kinin, kallidin, bradykinin) or vasodilator (kallikrein) principles.

Four healthy men, aged 20–23 years, were injected intravenously with 10 ml. of a 5 per cent sterile solution of Evans blue in saline. Intradermal injections (0·05 or 0·1 ml.) of the test substances in saline were made into the skin of the back 30 min. later, and flares and weals were measured 8–15 min. after injection. Measurements were made either directly on the subject or from projected coloured photographs. The mean of the largest diameter and that at right angles to it was taken as the size of the flare or weal; the flare measurement included the diameter of the weal. Serial photographs of the skin reactions indicated that the flare usually reached its maximum in 4 min. and was beginning to fade in 15 min. The weal reached its maximum size in 9 min. and remained so beyond 30 min. Reactions were measured, therefore, 8–15 min. after the intradermal injection. Small weals that were not blue were designated as 0 or 0–4, since they presumably represent injected fluid and not fluid which had left the circulation. These were never, in any event, greater than 4–5 mm. in diameter.

Wasp kinin was prepared by extraction of wasp glands with glacial acetic acid, precipitation with ether, and repeated washing of the precipitate with ethanol. This preparation contained 1 unit in 0·4 μgm. of freeze-dried material. Kallidin and bradykinin were prepared by the action of human salivary kallikrein or trypsin, respectively, on heated ox globulin. These preparations were of equal potency and contained 1 unit in 75 μgm. dry material in terms of the unit of Rocha e Silva, Beraldo and Rosenfeld[5].

Details of the units and preparation of these different substances are given by Holdstock *et al.*[3]. Kallikrein was prepared from the saliva of the subject in whom it was eventually tested. There is as yet no standard of kallikrein in terms of its ability to release kallidin; the preparation used in these experiments, however, released detectable amounts of kallidin when incubated with heated human plasma in concentrations of 10 μgm./ml. or less.

The results obtained with intradermal injections of 0·05 ml. kinin, histamine and 5-hydroxytryptamine in three subjects are shown in Table 1. Histamine and partially purified wasp kinin produced marked blueing with a large surrounding flare and were approximately equi-active on a weight basis. 5-Hydroxytryptamine produced a flare, but no blueing, even in much higher concentrations. The small, evanescent flare produced by saline was not measurable 5 min. after the injection.

Preparations of bradykinin and kallidin which were of equal activity on the guinea pig ileum were compared with kallikrein, histamine, kinin and 5-hydroxytryptamine in a fourth subject (Table 2). In this experiment, a greater volume, namely, 0·1 ml., of each solution was injected. Kallidin and bradykinin were effective blueing agents but much less active in producing flare. Thus, unlike histamine and wasp kinin (Table 1), they were still producing blueing reactions without an associated flare in high dilutions. Kallikrein, which produced larger weals, was also practically devoid of flaring properties in the concentrations used. 5-Hydroxytryptamine, on the contrary, again elicited a strong flare but no blueing reaction.

Table 1. FLARE AND WEAL PRODUCED BY INTRADERMAL INJECTIONS (0·05 ML.) OF KININ, HISTAMINE AND 5-HYDROXYTRYPTAMINE (Combined results from three subjects)

Substance	Concentration (μgm./ml.)	Mean diameter (mm.)	
		Flare	Weal (blue area)
Wasp kinin	500	48, 44, 43	15, 14, 10
	50	48, 44, 35	13, 10, 9
	5	32	9
Histamine	500	55, 51	16, 14
	100	47	13
	50	48, 41	10, 10
	10	43	9
	1	30	9
5-Hydroxytryptamine	2,500	35, 32	0–4, 0–4
Isotonic saline		0, 0	0–4, 0–4

Table 2. FLARE AND WEAL PRODUCED BY INTRADERMAL INJECTIONS (0·1 ML.) OF BRADYKININ, KALLIDIN, KALLIKREIN, HISTAMINE AND 5-HYDROXYTRYPTAMINE IN A FOURTH SUBJECT

Substance	Concentration (μgm./ml.)	Mean diameter (mm.)	
		Flare	Weal (blued area)
Bradykinin	1,000	30	15
	333	27	13
	100	0	12
	10	0	10
Kallidin	1,000	28	15
	333	20	9
	100	0	11
	10	0	9
Kallikrein	500	trace	18
	50	0	15
Kinin	100	52	18
Histamine	100	58	20
5-Hydroxytryptamine	100	34	0–4
Isotonic saline		0	0–4

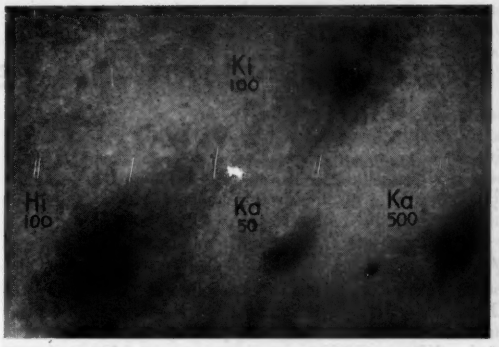

Fig. 1. Photograph of weal and flare reactions of human skin to intradermal injections of 0·1 ml. of histamine (*Hi*), wasp kinin (*Ki*) and salivary kallikrein (*Ka*). The numbers represent the concentration of the solutions in μgm./ml. The subject had received Evans blue solution previously, and the blue weal is the dark central area surrounded by the lighter flare. Note the complete absence of flare with kallikrein

Fig. 1 shows the flare and weal produced by histamine and wasp kinin contrasted with the pure wealing action of kallikrein.

These results indicate that in addition to histamine a number of naturally occurring substances produce wealing in man. They include wasp kinin, salivary kallikrein, kallidin and bradykinin. 5-Hydroxytryptamine, which increases capillary permeability in rat skin[4,5], has no such effect in man, where, however, it is effective in producing the local flare reaction. It is very unlikely, therefore, that the release of 5-hydroxytryptamine is of significance in reactions involving wealing or angio-œdema in man.

The fact that salivary kallikrein and the polypeptide, kallidin, which it releases are effective in increasing capillary permeability is of interest, because plasma contains large amounts of kallikrein in an inactive but activable form[7]. Indeed, there is a suggestive similarity in the blueing of skin produced by salivary kallikrein in our experiments and that produced by diluted human plasma in man[8]. In both instances flare reactions were strikingly absent. It may well be, therefore, that the agent in diluted human plasma which increases permeability is kallikrein, since there is also evidence that dilution of plasma or serum activates kallikreinogen, the precursor of serum kallikrein[9].

In our experiments kallidin itself did produce some flare reaction, although this was much less prominent than with histamine, kinin, or 5-hydroxytryptamine. In higher dilutions kallidin no longer produced any flare although it still caused blueing. Our preparations of kallidin and bradykinin are quite impure, and until pure preparations are available for testing, it will be impossible to say whether the mild flare reaction is produced by the same substance which produces the increase in capillary permeability.

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CELLULOSE ACETATE AS A MEDIUM FOR IMMUNO-DIFFUSION

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A NUMBER of well-established methods for demonstrating antibody–antigen reactions depend on the formation of precipitin lines by diffusion through a jelly—usually agar. Some of the difficulties associated with the use of jelly would be avoided if the diffusion process could occur in a sheet of solid material. For satisfactory results such a material would have to be wettable, allow the free diffusion of large molecules without adsorption, have a uniform composition and be readily available. We have found that cellulose acetate membrane (Oxo, Ltd.) fulfils these requirements and is equal, if not superior, to agar jelly for immuno-diffusion purposes. This material, in strip form, was introduced by Kohn[1] for electrophoresis and was later developed, in conjunction with agar, for immuno-electrophoresis[2,3].

For the Ouchterlony–Elek double-diffusion method, pieces of suitable size are cut from a membrane strip or, alternatively, unprinted circular membrane filters may be used. The positions where the samples are to be applied are marked lightly with non-diffusing ink (for example, with a ball-point pen). The membrane is then soaked in the desired buffer solution, blotted lightly so that no excess moisture is visible and supported in a horizontal position in a moist chamber. (A suitable support consists of pins with their heads pressed into a 'Plasticine' base so that the points are uppermost.) By means of capillary tubes, small amounts (1–5 μl.) of the samples are applied as spots or lines to the membrane surface. Antigen and antiserum are usually placed 1 cm. apart, but the arrangement and distance may be chosen as required. The membrane is allowed to remain in the moist chamber until the drops have sunk into the surface, following which it is immersed in a bath of liquid paraffin or 'Whitemor 120' oil (Manchester Oil Refinery Co.). Diffusion occurs in the moist membrane and the formation of precipitin lines is usually complete in 2 days at room temperature. Excess of oil is wiped off, and the remaining oil removed by a quick wash in ether or light petroleum. Thereafter, water-soluble material is removed by soaking in saline or buffered saline solution for 1–3 hr. with occasional agitation. The membrane is then blotted and stained to render the precipitin lines visible and finally dried flat between filter paper sheets at room temperature. The stains used were ponceau *S* (G. T. Gurr) (0·15 per cent in 3 per cent trichloracetic acid) for strong precipitin lines and dilute nigrosine *WS* (0·001–0·0025 per cent in 2 per cent acetic acid) for weaker lines as described by Kohn[3]. The membrane may be stained first with ponceau then with nigrosine. Examples of double diffusion on membranes are illustrated in Figs. 1*a* and 2*a*. Fig. 1*b* shows a conventional agar plate diffusion employing the same samples of antiserum and antigens as in Fig. 1*a*.

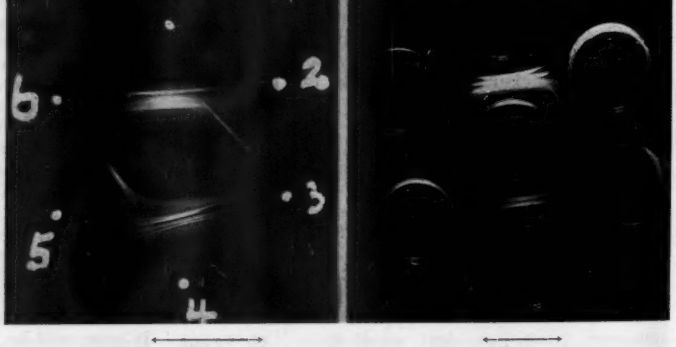

Fig. 1. (*a*) Double diffusion on cellulose acetate membrane at *p*H 7 for 2 days. Human serum or human serum fractions (2–3 μl.) placed at positions 1–6, horse anti-human serum at centre. Stained with ponceau *S*. Negative print from oil transparency. (*b*) Diffusion on agar with same samples (about 0·3 ml. in each well)

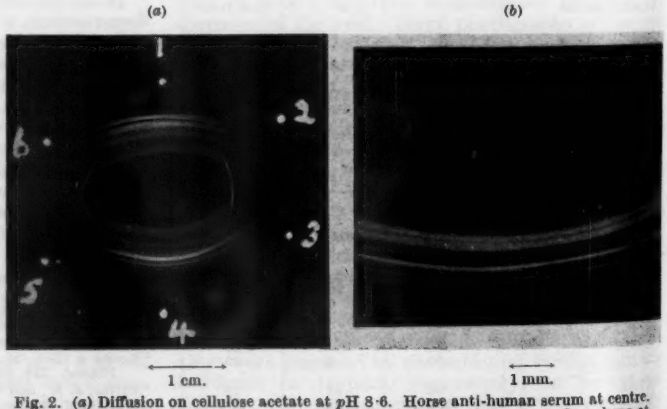

Fig. 2. (*a*) Diffusion on cellulose acetate at *p*H 8·6. Horse anti-human serum at centre. Normal human sera at positions 1 and 4. Concentrated urines from cases of myeloma at positions 2, 3, 5, 6. Stained with ponceau *S*. (*b*) Portion of membrane above position 4 in (*a*) enlarged direct from transparency

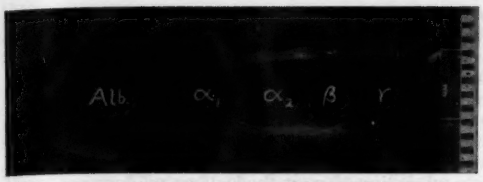

Fig. 3. Immuno-electrophoresis on cellulose acetate membrane (12 cm. × 2½ cm.). Electrophoresis of normal human serum (3 μl.) carried out at 20 V./cm. for 1½ hr. in barbitone buffer, *p*H 8·6. Horse anti-human serum placed along membrane above and below antigens after electrophoresis. Stained with nigrosine. Negative print from transparency

The stained membrane is best viewed in transmitted light, and may be photographed in this way. However, when soaked in 'Whitemor' oil, cellulose acetate, when dry, becomes almost transparent. The oiled, stained membrane if sandwiched between glass plates may then be used as a transparency for projection or for enlargement in a photographic enlarger. An enlargement of a small portion of a membrane is shown in Fig. 2*b* and demonstrates the fine nature and sharpness of the precipitin lines.

The Oudin-type of diffusion is carried out on a strip of cellulose acetate. Antigen and antiserum are each applied across the width of the strip about ½ in. apart. The strip is hung vertically in the oil during the diffusion stage.

For immuno-electrophoresis, the electrophoresis stage is carried out on the membrane strip as described by Kohn[1,2], the sample (1–5 μl.) being applied as a drop or across a short line at the starting position. After the electrophoresis, and without removing the strip from the box, a line of antiserum is applied from the capillary, preferably in one movement, parallel to the electrophoresis direction and at a distance of 0·5–1 cm. from the antigens. The lid is then replaced until the serum has soaked in, when the strip is removed to the oil bath. Subsequent procedures are as described above. Fig. 3 illustrates an immuno-electrophoretic pattern.

In these experiments diffusion under oil has been preferred to diffusion in a moist atmosphere, because in the latter conditions it is difficult during a long period to avoid unequal evaporation from the membrane surface which might cause artefact or distorted lines. The inert oil does not appear to have any denaturating effect on proteins.

Certain precautions should be observed in these techniques. No portion of the membrane should be allowed to become dry before transference to the oil bath. Should this occur, the membrane can be returned to the moist chamber and exposed until uniformly damp. All solutions and samples should be free from particulate matter, and the former should preferably contain a preservative to prevent bacterial growth; colonies of micro-organisms may cause distortion of precipitin lines and form an undesirable patchy background when stained. Membranes should be handled with forceps.

Cellulose acetate membranes have a number of advantages over agar. The material is relatively simple to use and satisfactory results are obtained with about 1/100 of the amounts usually used for gel diffusion (Fig. 1). In a membrane, precipitin lines can occur at the position of application of the samples but cannot form in the wells of an agar plate (Fig. 1). There is no difficulty in storing membranes. In jelly, of course, formation of the precipitin lines can be seen directly, whereas in a membrane they are not visible until stained. However, replicate diffusions may readily be carried out on membranes to establish optimum conditions, and, moreover, the material can be treated with a variety of dyes and reagents to obtain information about the chemical nature of the antigen in the precipitin lines. Cellulose acetate membranes are manufactured of standardized material in Britain, but a cellulose nitrate membrane has been found to be satisfactory for diffusion though not for electrophoresis. It is probable that other existing membranes may also prove suitable for one or both purposes.

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ABSORPTION OF ALKALI CATIONS BY PLANT TISSUE CULTURES

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It was observed by Steward and Millar[1] that actively growing carrot and artichoke tissue cultures absorbed cæsium ions more slowly per unit of fresh weight than did slowly growing cultures, and they concluded that, in general, the rate of uptake of salt is more rapid in vacuolated than in meristematic cells.

There were certain features of the experimental procedure which make it difficult to be sure that the differences observed were really due to the different physiological condition of the two types of tissue culture, as Steward and Millar suggested. Another possibility which occurred to us is that differences in the ionic composition of the two media employed may have been responsible for the effect. Although the concentrations of cæsium ions supplied were the same for each tissue (10^{-3} *M* and 10^{-5} *M*), the media were different with respect to the concentration of other ions because of the addition of 10 or 15 per cent coconut milk as a stimulant to the rapidly growing cultures. A sample of coconut milk which we analysed recently contained about 0·045 *M* potassium and 0·015 *M* sodium. The medium in which the slowly growing tissues were cultured contained about 0·0017 *M* potassium and 0·003 *M* sodium, and so it is evident that the composition of the two media, at least with respect to these ions, was quite different. Now it is well known that alkali cations compete with one another for absorption by plant tissues[2–4], and consequently it is to be expected that uptake of cæsium would be depressed by the presence of potassium and sodium; and to the greatest extent in the actively growing cultures where the concentrations of potassium and sodium in the medium were highest.

In order to test this possibility, we have repeated some of Steward and Millar's experiments using carrot explants placed in media with and without 10 per cent coconut milk, but with the initial potassium

Table 1. Cæsium Content (μequiv. per gm. fresh weight), and the Fresh Weight (gm.) of 3 Replicate Batches of 10 Cultures of (*A*) slowly growing and (*B*) actively growing Carrot Tissues, after 2 and 3 weeks at 23° C.

Treatment		After 2 weeks		After 3 weeks	
		Fresh wt.	Cs content	Fresh wt.	Cs content
A	1	0·086	1·3	0·147	1·4
	2	0·098	1·1	0·137	1·7
	3	0·083	1·5	0·121	1·7
B	1	0·240	0·76	0·560	0·51
	2	0·245	0·66	0·608	0·52
	3	0·245	0·80	0·576	0·51

and sodium concentrations adjusted to the same value in both media (0·01 *M* for each ion) by addition of appropriate amounts of potassium and sodium chlorides. Cæsium chloride, labelled with cæsium-134, was supplied in each solution at a concentration of 10^{-4} *M*. The results obtained are shown in Table 1.

It was observed that although the actively growing cells absorbed more cæsium per culture than the slowly growing tissues, they absorbed considerably less on a gm. fresh weight basis. The differences observed were much lower than those found by Steward and Millar, who reported a 14-fold difference in uptake per unit of fresh weight between the two kinds of cultures. It must be concluded that the bulk of the effect observed by these workers is attributable to the composition of the media they used, rather than to the differing absorptive abilities of rapidly growing and slowly growing tissues; nevertheless the growing cultures seemed in our experiment to be somewhat inferior in their accumulatory capacity.

It seemed to us important to establish that the last conclusion is a general one, applying to physiologically important cations as well as to cæsium. Therefore, in another similar experiment we measured the uptake of potassium and sodium ions by carrot tissues grown in culture media containing each of these ions at a concentration of 0·01 *M* in the presence and absence of coconut milk.

Table 2. Potassium (K) and Sodium (Na) Content (μequiv. per gm. fresh weight), and the Fresh Weights (gm.) of (*A*) slowly growing and (*B*) actively growing Carrot Tissue Cultures, in 2 and 3 weeks at 23° C.

	After 2 weeks		After 3 weeks	
	A	*B*	*A*	*B*
	(*S.E.*)	(*S.E.*)	(*S.E.*)	(*S.E.*)
Fresh wt.	0·075±0·006	0·262±0·018	0·103±0·006	0·536±0·005
K	104±7·8	123±5·0	128±12·1	123±3·7
Na	68±7·1	15·6±1·0	70±4·7	21·5±1·1
Ratio K/Na	1·5	7·9	1·8	5·7

The results of this experiment (Table 2) show that untake of potassium per gm. fresh weight was not significantly different between the two treatments. Uptake of sodium, however, was considerably higher in the slowly growing than in the actively growing tissues. The ratio K/Na in the tissue remained fairly constant over the experimental period in the case of slowly growing tissues, but decreased with time in the actively growing cultures. It is clear from these results that actively growing cells exhibit a stronger preference for potassium over sodium than do slowly growing ones, but that the difference becomes less marked with time. One possible explanation is that in actively growing tissues, containing a higher proportion of meristematic and nearly meristematic cells, relatively more of the absorbed ions are retained by the cytoplasm than in the slowly growing, mainly vacuolated cells where the bulk of the salt is transferred to the vacuoles. Retention in the cytoplasm may depend to some extent on ion-binding to specific sites (for example, on enzymes) which exhibit a marked potassium preference, whereas vacuolar accumulation in carrot cells may be relatively unselective with respect to sodium and potassium. In support of the last statement we have found that the prolonged metabolic accumulation of cations by washed slices of dormant carrot tissues, which is presumably mainly vacuolar accumulation, does not exhibit any marked discrimination between potassium and sodium. By the end of 3 weeks culture, the rate of growth in the actively growing tissue was beginning to decrease, and this was probably accompanied by increased vacuolation, which may account for the reduction in the ratio K/Na absorbed with time.

In an attempt to distinguish more clearly between cytoplasmic and vacuolar accumulation in tissue culture cells, we have studied the uptake of potassium and sodium by growing red beet cells in which it is already known that vacuolar accumulation exhibits a preference for sodium over potassium[4]. Explants were cultured in three different media: (*A*) a basal medium containing salts and sugar; (*B*) basal medium + indole-3-acetic acid (5×10^{-5} *M*); (*C*) basal medium + 10 per cent coconut milk. In all treatments the concentrations of potassium and sodium were adjusted initially to 0·01 *M*. The tissues grew appreciably during the experimental period although not so rapidly as carrot tissue cultures. Microscopic examination indicated that the growth in *A* and *B* was mainly, if not entirely, by cell enlargement; whereas the growth in *C* was predominantly the result of cell division accompanied by a little cell expansion.

Table 3. Potassium (K) and Sodium (Na) Uptake (μequiv. per gm. fresh weight) and the Fresh Weights (gm.) of Beet Tissues after 2 weeks of Culture at 23° C. in (*A*) Basal Medium; (*B*) Basal Medium + Indole-3-acetic acid; (*C*) Basal Medium + Coconut Milk

		A	*B*	*C*
Fresh wt.		0·044	0·081	0·079
Uptake	K	17	26	79
	Na	161	126	94
Ratio K/Na		0·11	0·21	0·84

Salt analyses showed (Table 3) that tissues in the various media absorbed sodium in excess of potassium during the experimental period. Tissues in the medium containing coconut milk, however, showed a much smaller preference than did the tissues cultured in the other media, and this might be attributable to a partial masking of the sodium-preferring vacuolar accumulation by concomitant cytoplasmic accumulation exhibiting a preference for potassium.

These results, therefore, are consistent with the idea of a two-phase metabolic accumulation of cations into: (*a*) cytoplasm and (*b*) vacuoles of plant cells. In both beet and carrot tissue cultures the two components could be distinguished on the basis of selectivity, and it is of interest that cytoplasmic accumulation apparently exhibited a potassium preference even when vacuolar secretion was characterized by a strong preference for sodium. Possibly this is related to the metabolic utilization of potassium rather than sodium by the cells.

A full account of this work, which is now being extended, will be published elsewhere in due course.

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LETTERS TO THE EDITORS

PHYSIOLOGY and BIOCHEMISTRY

The Work Output of Animals: Flight by Birds and by Man-power

MEN have always wanted to fly by their own power, like birds. But man is five times heavier than the heaviest flying creature of this or any other epoch. This creates difficulties; for as birds increase in size, their wing area diminishes in relation to their weight, since geometrical configuration and density remain roughly the same. This effect can only be countered by a greater expenditure of mechanical power by the larger specimens. In fact, the power required for level flight increases as (weight)$^{1\cdot167}$ (Nonweiler, 1957, private communication).

Imagine a set of flying machines having the same shape and density, but differing in size. Let L be a characteristic length, and let V be the forward speed: then both lift and drag are proportional to V^2L^2. To achieve flight, weight = lift. Thus $L^3 \propto V^2L^2$, so that $V \propto L^{0\cdot5}$. Power expended = speed × drag $\propto V.V^2.L^2 \propto L^{3\cdot5} \propto$ (weight)$^{1\cdot167}$.

Successful aeroplanes have been built that range in size from a few grams up to nearly 200 tons. This is possible because artificial sources of power are available which provide considerably more power than is needed merely to achieve flight. Internal-combustion aero-engines are available with a weight range of 1 : 10^6, and they all produce about 1·5 horsepower per kilogram. Full-sized engines actually become more powerful as they become bigger; their power goes up roughly as their (weight)$^{1\cdot28}$.

But what of living creatures? What is their power output, and how does it vary with their size?

The Power Output of Animals is small compared with that of petrol engines of similar weight. For example, the maximum continuous power output of an athletic man is only 0·5 per cent of the output of an engine of comparable weight.

One of the best-established facts of comparative physiology is that the resting metabolism of animals varies with their size in an exceedingly regular manner. The smaller the animal, the greater its resting metabolism per kilogram. This is illustrated in line *A* of Fig. 1. The black spots represent actual measurements of the resting energy output of dogs, men, and horses. They are extracted from the much larger series of measurements on mammals and birds (from mice to elephants, a range in weight of more than five decades) collected by Brody (ref. 1, pp. 370, 388). He showed that these measurements could be fitted by the equation:

resting metabolism in h.p. = $4\cdot6 \times 10^{-3}$ (weight in kgm.)$^{0\cdot734}$

Line *A* is drawn from this equation.

Now, an animal at rest is doing no external work, its energy output is all in the form of heat. We want to know what happens when it *is* performing external mechanical work. This is not easy to investigate experimentally. Most animals can be lulled to rest, but few can be induced to work steadily and energetically. Moreover, the mechanical work must be performed in such a way that it can be measured. The final difficulty, which is almost psychological, is that a valid comparison can only be made between animals that are trying equally hard to perform comparable tasks.

The only measurements that I have been able to find in which all these criteria were satisfied were on dogs climbing a treadmill[2] or pulling a sledge[3]; men cycling, rowing, climbing, etc; and horses pulling[1].

In each case it is possible to fit a reasonably good straight line to the points which is parallel with line *A*. This shows that the output of mechanical work varies with size in exactly the same way as does the resting metabolism, and helps to support Brody's claim that one can express the effort that an animal is exerting as a multiple of its resting metabolism. Lines *B*, *C* and *D* correspond to mechanical power outputs of 1·7, 4·8 and 25 times basal heat production. Because of the relative inefficiency of muscular contraction, the total production of energy (work + heat) resulting from exercise will be about four times greater, say, 7, 20 and 100 times basal. The conclusion to be drawn is that the maximum metabolic rate is linked to the resting metabolic rate; though it is not clear what the physiological mechanism of this link may be.

We are now in a position to appreciate the dilemma of the birds that tended, in the course of evolution,

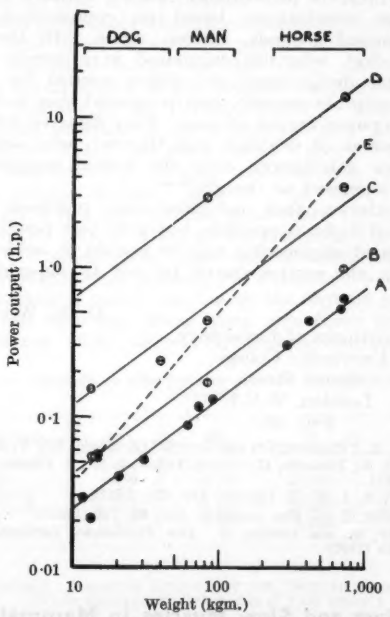

Fig. 1. Power output of animals as a function of body-weight; plotted on a double logarithmic scale. *A* (black spots), heat output at rest; *B*, *C*, *D* (circles), work output at various levels of effort; *B*, maintained all day; *C*, maximum steady-state (maintained 5–30 min. approximately); *D*, brief effort (maintained a few seconds only, by going increasingly into oxygen debt). The full lines have been fitted from the equation: horse-power = const. 10^{-3} (kgm.)$^{0\cdot734}$, where const. is 4·6, 7·4, 21 and 113 respectively; *E* (dashed line) shows the power required to fly, calculated from the relation:

horse-power = $2\cdot33 \times 10^{-3}$ (kgm.)$^{1\cdot167}$.

• Intersection of *B* and *E* represents the great bustard.

to become larger. As they did so their power production (lines *B*, *C*, *D*) became relatively less, while their power requirement (line *E*) increased. Evidently flying ceased to be a practical proposition for birds heavier than about 15 kgm. This is not to say that it is impossible; merely that it is too expensive a means of transportation for a bird in a competitive world.

Line *E* has been drawn with the theoretical slope derived from the relation:

$$(\text{power required}) = \text{const.}\ (\text{weight})^{1.167}$$

but in order to fix the position of the line it is obviously necessary to estimate the value of the constant. This involves estimating, or guessing, the power output of a bird of known weight. I have chosen the great bustard, because, at 14·5 kgm. it is the heaviest flying bird. It is said to be capable of powerful and sustained flight, so I have assumed that it can fly with its 'all-day' level of effort (line *B*) and have accordingly made line *E* intersect *B* at 14·5 kgm.

Fig. 1 then leads to the interesting conclusion that it should be possible for birds weighing up to 170 kgm. to fly while still remaining in oxygen balance, though they would have to work extremely hard to do so. Line *E* passes just below the maximum steady-state point for a man; so if he had wings he would be able to fly. Unfortunately, in practice, flying machinery will have to be carried as an extra—the extra weight per man (assuming a two-man machine) can scarcely be less than 40 kgm. This may mean that man can only fly by incurring an oxygen debt, which would limit the performance to a few minutes.

These conclusions, based on comparative and dimensional grounds, entirely agree with those of Nonweiler[4], who has calculated in a purely aerodynamic design-study the power needed for flight by a suitable aircraft, and compared this with the known power output of man. They disagree with the conclusions of Günther and Guerra[5], who assumed in their calculations that the power required for flight increased as $(\text{weight})^{0.667}$.

Calculation thus indicates that practical man-powered flight is possible, but only just possible, so that good engineering will be needed to achieve it. Clearly, the matter should be put to experimental test.

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Fast and Slow Muscles in Mammals

The histology and physiology of both fast and slow types of cat striated muscle were studied in detail by Denny-Brown[1]. He reported that the histological appearance did not distinguish definitely between the functionally fast and functionally slow type of muscle fibre. From his physiological investigations he concluded that the typically fast muscles (for example, gastrocnemius) were specialized for phasic contractions, whereas the typically slow muscles (for example, soleus) were utilized for tonic contractions. He suggested that a comparable arrangement of fast gastrocnemius and slow soleus might be found in many animals, including man.

The renewed interest in the physiology of fast and slow contracting muscles[2] has prompted an attempt to measure the contraction times in human muscles. The responses of the gastrocnemius and soleus muscles were examined in sixteen normal males between the ages of 18 and 40 years, some repeatedly. The subjects were examined while lying at rest in the prone positions. The muscle contraction was recorded by means of a saline-filled needle inserted into the belly of the muscle and connected to a capacitance manometer measuring the pressure among the fibres. The muscle fibres lying adjacent to the recording needle were caused to contract by an electrical stimulus applied either via the recording needle itself, or via a second needle placed in close proximity to the first. Thus the pressure changes within a group of contracting muscle fibres were measured. When this technique was used in the muscles of the cat and the results compared with simultaneous measurements of tension changes using the conventional strain gauge, it was found that, although it did not accurately record the time-course of the contractile process, nevertheless the method adequately differentiated the fast and slow muscles of the cat as shown in Fig. 1.

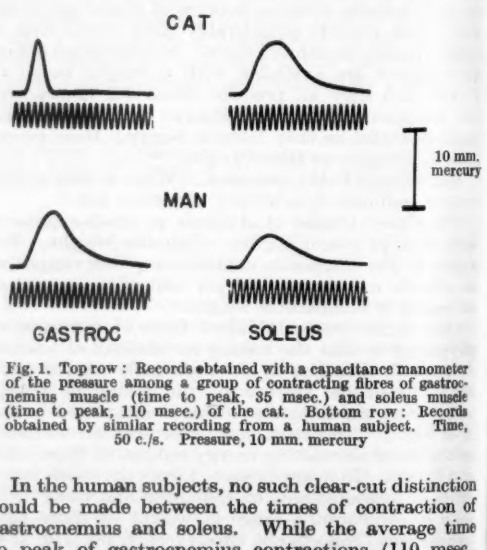

Fig. 1. Top row: Records obtained with a capacitance manometer of the pressure among a group of contracting fibres of gastrocnemius muscle (time to peak, 35 msec.) and soleus muscle (time to peak, 110 msec.) of the cat. Bottom row: Records obtained by similar recording from a human subject. Time, 50 c./s. Pressure, 10 mm. mercury

In the human subjects, no such clear-cut distinction could be made between the times of contraction of gastrocnemius and soleus. While the average time to peak of gastrocnemius contractions (110 msec., range 92–150 msec.) was somewhat shorter than that of soleus (120 msec., range 100–150 msec.), no regular pattern emerged, and in any one subject the times of contraction of the two muscles often differed by less than 20 msec.

In preliminary studies of human muscles other than those of the calf (forearm and hand), it was noted that considerably shorter times of contraction than with soleus and gastrocnemius could be obtained, but so far none shorter than 45 msec. to peak.

It therefore appears that, contrary to Denny-Brown's suggestion, the clear-cut differentiation of gastrocnemius and soleus into fast- and slow-con-

tracting muscles respectively does not occur in the human.

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Effect of Monofluorosulphonate, Difluorophosphate and Fluoroborate Ions on the Iodine Uptake of the Thyroid Gland

Several inorganic anions have been found to interfere with the normal function of the thyroid gland[1-3], the perchlorate and thiocyanate ions being outstanding in this group. On the other hand, it has been shown that other common anions, including chloride, bromide, fluoride, sulphate, phosphate and bromate ions, have no significant effect on the iodine uptake and retention in the gland. The effect of perchlorate ions suggests a competition for some sites in the thyroid[1]. This assumption is supported by the fact that perchlorate ions concentrate in the gland[4]. Further, it was shown[4] that perchlorate ions do not undergo metabolization, thus excluding the possibility that they are involved in some enzymatic redox system. This behaviour of the perchlorate ion distinguishes it from other inorganic thyroid blocking agents like thiocyanate ions which have been shown to be metabolized and not to accumulate in the gland[2]. It occurred to us that the competition between perchlorate and iodide ions may be due to their similar monovalency and size. It is hard to compare the size of a spherical ion like iodide with that of a tetrahedral complex ion like perchlorate. Still we may compare the calculated volumes. The volume of ClO_4^- may be calculated taking $r(O^=) = 1{\cdot}33$ A. From the radius of I^-, $r(I^-) = 2{\cdot}16$ A., we may find its volume. We find for the volumes of ClO_4^- and I^- the values $3{\cdot}94$ and $4{\cdot}23 \times 10^{-23}$ cm.^{3} respectively. The experimental value[6] for the ionic volume of ClO_4^- is $4{\cdot}05 \times 10^{-23}$ cm.^{3}.

In order to check the hypothetical correlation between ionic size and its effect on the thyroid function, three ions which are isoelectronic with and of almost the same size as ClO_4^- were chosen. The similarity between perchlorate, monofluorosulphonate, difluorophosphate and fluoroborate ions has been pointed out[6,7]. The similarity of the ionic sizes of these ions may be deduced from the values of the bond-lengths in ClO_4^- (r(Cl—O) = 1·50 A.)[6], in sulphate (r(S—O) = 1·50 A.)[6], in phosphate (r(P—O) = 1·50 A.)[6] and in fluoroborate (r(B—F) = 1·48 A.)[3], and, on the other hand, from the fact that[5] $r(O^=) = r(F^-)$. In our experiments we compared, under identical conditions, the effects of the three ions mentioned above with that of perchlorate on the accumulation of iodine in the thyroid.

Monofluorosulphonate and difluorophosphate were synthesized by the reaction of ammonium fluoride with sulphur trioxide and phosphorus pentoxide respectively[9]; they were separated and purified by precipitation of their corresponding nitron complexes and finally their ammonium salts were recrystallized. Potassium fluoroborate was prepared from commercial fluoroboric acid and was recrystallized before use.

0·1 ml. of a 1 per cent solution of propylthiouracyl was injected into young rats (100–110 gm.) intraperitoneally, in order to block the formation of protein-bound iodine in the gland[3]. 1 hr. after this injection, 0·1 ml. of carrier-free iodine-131 iodide solution (40 mc./ml.) was injected. 15 min. later an injection of 0·5 ml. saline containing 25 μmoles of $KClO_4$, NH_4SO_3F, $NH_4PO_3F_2$ and KBF_4 respectively was given. The control rats were injected with 0·5 ml. of pure saline. The rats were killed at intervals of 30, 60, 90 and 120 min. after the iodide injection. The whole thyroid gland was removed and 1 ml. fresh blood was measured volumetrically and taken as a reference. The activity of the thyroid glands and of the blood samples was determined in a well-type sodium iodide scintillation counter. A summary of our experimental results is presented in Tables 1 and 2.

From the experimental results it is evident that all four ions tested are effective at these low concentrations. The increase in uptake with time of the $PO_3F_2^-$ series is most probably due to the hydrolysis of this ion[9] to F^- and $PO_4H^=$, which are not effective as inhibitors of iodide uptake[1]. It should be noted that these thyroid blocking agents have an effect within 15 min. after injection.

Other substances which accumulate in the thyroid, and thus interfere with its normal function, have been described lately, namely, TcO_4^- and ReO_4^-. Their accumulation in the thyroid was attributed to their position in the Periodic Table[10]. Evaluating the ionic volumes of these ions, we find[6] that the values for MnO_4^- and ReO_4^- are $4{\cdot}0$ and $4{\cdot}1 \times 10^{-23}$ cm.^{3} respectively; we may therefore infer that the ionic volume of TcO_4^- is $4{\cdot}05 \times 10^{-23}$ cm.^{3}, which actually is the experimental value found for the ionic volume of ClO_4^-. Thus we may include TcO_4^- and ReO_4^- in our series of effective monovalent anions.

It is worth while pointing out a correlation between the uptake of certain anions by the thyroid proteins and the fact that these very ions form insoluble complexes with nitron (1,4-diphenyl-3,5-endanilo-

Table 1. Activity of the Thyroid Glands (Counts/Min./Whole Thyroid)

Time (min.)	30	60	90	120
Control	78,670	129,270	83,840	71,350
$KClO_4$	8,420	8,630	10,150	6,740
NH_4SO_3F	33,500	8,200	23,050	22,440
$NH_4PO_3F_2$	20,630	7,220	17,850	58,670
KBF_4	8,480	8,200	6,250	9,620

Table 2. Relative Activity of the Thyroid Glands
(Whole thyroid activity/activity of 1 ml. blood*)

Time (min.)	30	60	90	120
Control	1·80	4·45	3·20	2·90
$KClO_4$	0·15	0·13	0·26	0·34
NH_4SO_3F	0·70	0·38	1·00	1·09
$NH_4PO_3F_2$	0·45	0·24	0·66	2·16
KBF_4	0·11	0·11	0·14	0·29

* Taking the average weight of a rat's thyroid as 12 mgm. we may calculate the relative specific activity of the thyroid by multiplying the relative activity of the whole thyroid given in Table 2 by 833

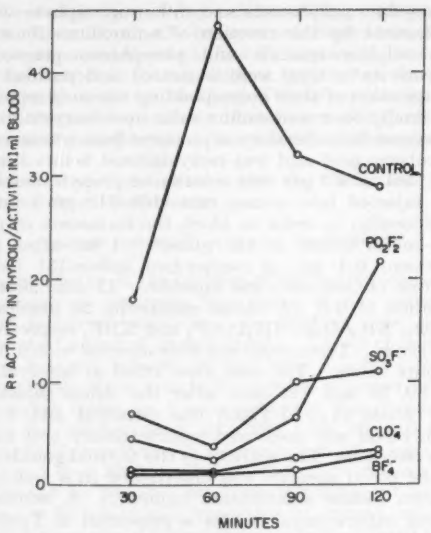

Fig. 1. Relative activity of thyroid glands of rats injected with inorganic blocking agents

4,5-dihydro-1,2,4-triazole). These ions include not only perchlorate and its isoelectronic species but also iodide, thiocyanate, chlorate and nitrate; all of them were found to take part in the function of thyroid[1]. The striking fact is that whereas chlorate has some effect on iodide uptake, no such effect was found for bromate[1]; it has been shown that iodate does not accumulate in the thyroid[11]. It has been shown[12], moreover, that nitron does not form insoluble complexes of appreciable stability with iodate and bromate.

It may be concluded that the trapping mechanism in the thyroid involves a factor which scarcely distinguishes between different anions having the same charge and a comparable volume. Such a factor is probably a protein with a special arrangement which traps only those ions which fulfil these requirements.

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In Vitro Effects of Insulin and Glucose on Leucine-2-^{14}C Incorporation into Protein of Diaphragms of Hypophysectomized Rats

Insulin, added *in vitro*, has been found to stimulate the incorporation of labelled amino-acids into the protein of isolated diaphragms of normal rats[1–4]. Glucose, when added to the incubation medium, depressed the stimulatory action of insulin on the uptake of alanine labelled with carbon-14 [1,3] and α-amino*iso*butyric acid-^{14}C [4] by normal diaphragm. Even when insulin was not added to the incubation medium the uptake of these amino-acids was markedly depressed by supplementing the medium with glucose. In one report, however, the incorporation of glycine labelled with carbon-14 into protein of diaphragms of normal fasting rats was found to be increased by the addition of glucose alone to the medium[2]. A later study[3] failed to confirm this observation and indicated further that neither glucose nor pyruvate seriously altered the uptake of a number of amino-acids labelled with carbon-14 by normal diaphragm. From the above studies it was apparent that insulin had a stimulatory effect on the uptake of amino-acids by isolated muscle of normal rats, and that in certain instances this effect was altered by the concomitant utilization of carbohydrate. Thus, it was of interest to study the effects of insulin and of glucose, alone and in combination, on the *in vitro* incorporation of labelled leucine by the diaphragm of hypophysectomized rats, which show a decreased rate of incorporation of amino-acid[5] but which utilize glucose at a greater than normal rate[6].

Female rats (60 gm.) that were hypophysectomized three weeks previously were killed by a blow on the neck, and the diaphragm was quickly excised with part of the rib cage and spine attached and placed in freshly gassed buffer. The diaphragm was then divided into two hemi-diaphragms by cutting through the central ligament and through the insertions to the xiphoid process and spine. Each hemidiaphragm with ribs attached was blotted and placed in a 50-ml. Erlenmeyer flask and incubated with shaking for 90 min. at 37° C. The incubation flasks contained 6 ml. Krebs–Ringer phosphate buffer, *p*H 7·4, to which DL-leucine-2-^{14}C was added at a concentration of 0·1 m*M* (25,000 c.p.m./ml. medium). Each flask was gassed with 100 per cent oxygen during the first 2 min. of incubation and then sealed. Insulin (Lilly, crystalline, No. *T*-2842) was added to the medium at a concentration of 0·5 U./ml. and glucose was added at a concentration of 2 mgm./ml. medium. The incorporation reaction was terminated by rapidly chilling the flasks to 0° C. The tissue was then spread on a block of ice and the diaphragm muscle carefully trimmed of ribs and adhering tissue. The protein of the diaphragm was prepared and counted by methods published elsewhere[5].

The results are presented in Table 1. Insulin, added *in vitro*, increased the incorporation of leucine-2-^{14}C into the protein of diaphragms of hypophysectomized rats. Glucose added alone to the medium did not alter the incorporation of leucine by the diaphragms. Another experiment (not given in Table 1) with glucose added alone was carried out over a range of incubation times of 30–150 min., and again glucose addition had no effect on incorporation of leucine. When glucose and insulin were added together to the medium, the incorporation of leucine by the protein of the diaphragms was increased, but

Table 1. *In vitro* EFFECT OF INSULIN AND GLUCOSE ON INCORPORATION OF LEUCINE-2-^{14}C INTO PROTEIN OF HYPOPHYSECTOMIZED RATS

Added to medium	No. of observations	c.p.m./mgm. protein	
		Mean	*S.E.*
Control	6	189	± 12·6
Insulin	6	287*	± 12·1
Glucose	6	186	± 22·4
Insulin + Glucose	6	280*	± 16·3

*Mean is significantly different from control, $P < 0.01$.

the increment was similar to that produced by insulin alone (Table 1).

These results indicate that insulin *in vitro* stimulated the incorporation of labelled leucine into the protein of diaphragms of hypophysectomized rats. The addition of glucose to the medium, in the presence or absence of added insulin, had no effect on this system. These results agree qualitatively with those obtained in experiments with normal rat diaphragms[3]; but the increase in leucine incorporation produced by insulin in the hypophysectomized rat diaphragm (151 per cent of control) was greater than that reported for diaphragms of normal rats (118 per cent of control). The lack of an effect of glucose on this system would favour the opinion expressed by Manchester and Young[3] that the action of insulin in stimulating amino-acid incorporation is largely independent of its action on carbohydrate metabolism.

It is of interest that, like insulin, chronic bovine growth-hormone treatment begun seven days post-operatively resulted in a stimulation of *in vitro* leucine incorporation by diaphragms of hypophysectomized rats[5]. It is possible that this stimulation of amino-acid incorporation produced after growth-hormone treatment was due in part to an elevated insulin activity in the isolated diaphragm resulting from an increased secretion of insulin brought on by growth-hormone treatment. This seems unlikely, however, since bioassays of plasma insulin activity have failed to detect an increase in insulin activity when growth-hormone treatment was given to hypophysectomized rats 7–9 days post-operatively[7]. Moreover, growth hormone injected into hypophysectomized rats 18–24 hr. before removal of the diaphragm lowers the insulin activity bound to this tissue, as assayed by glucose uptake[8].

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Effect of Norleucine and Related Compounds on Growth of the White Rat

IT was recently reported from this laboratory that the inclusion of 2 per cent DL-norleucine in the diet greatly inhibited the utilization of D-leucine by rats[1]. In view of this observation it was of considerable interest to determine whether the antagonistic effect of norleucine on leucine utilization was limited to the D-isomer of the latter.

The experiments summarized in Table 1 were designed to compare the effect of leucine isomers on the reversal of norleucine toxicity. Weanling albino rats of the Yale strain which were previously fed on a nitrogen-free diet for a 2-week period were used in this study. Following this period the animals were fed an amino-acid diet containing various levels of L- and D-leucine in the conjunction of 2 per cent DL-norleucine, as outlined in Table 1. The composition of the basal diet and the amino-acid mixture have been described elsewhere[2]. It can be seen from Table 1 that both enantiomorphs of leucine are antagonized by norleucine, but it is also apparent from the results that the antagonism is greater in the case of D-leucine. Although the exact quantitative relationship was not determined, it appears that at least twice as much of D-leucine is required to reverse norleucine toxicity when compared with the L-isomer. This is in good agreement with the previous observation that L-leucine is approximately twice as effective as the D-leucine for growth of young rats[3]. Note the close correlation between water consumption and growth.

Although norleucine does not seem to be a natural constituent of proteins[3] it is readily metabolized by mammals[4–7]. When incubated with rat liver homogenate it gives rise to α-ketocaproic acid, valeric acid and β-hydroxyvaleric acid. On the basis of such results, Kinnory *et al.*[8] concluded that norleucine is catabolized by conversion to the corresponding α-keto-acid, followed by oxidative decarboxylation and the subsequent oxidation to the resulting monocarboxylic acid by β-oxidation.

In order to learn more about the growth-depressing effect of norleucine an additional study was undertaken in which two of the above-mentioned intermediates of norleucine catabolism (α-ketocaproic and valeric acids) and two other compounds closely related to norleucine (caproic and ε-aminocaproic acids) were compared with norleucine for their effect on growth of rats. Since the growth-retarding effect of norleucine was previously demonstrated with the diet containing L-leucine at a level corresponding to 50 per cent of the requirement, that is, 0·43 per cent of the diet, it was decided to use this amount of L-leucine in our control diet. The compounds under test were added to the diet at molar equivalent levels corresponding to 1 per cent of norleucine. Normal weanling male albino rats of the Sprague–Dawley strain were used for assaying the above compounds. Each experiment covered 2 weeks. The experimental results which are shown in Table 2 demonstrate that of all the substances tested only norleucine and α-ketocaproic acid retarded the growth of rats. The slower rate of growth of animals was accompanied by lower consumption of food and decreased food efficiency. The inhibitory effect of the keto-acid is not unexpected since the first major step in the catabolism of most amino-acids, that is, formation of keto-acid, is readily reversible[9]. Since the keto-acid was less inhibitory than norleucine, while valeric acid

Table 1. Average Growth Response of Four Protein-depleted Weanling Rats to L- and D-Leucine in the Presence of DL-Norleucine

Supplement to leucine-free diet	Days	Initial weight (gm.)	Final weight (gm.)	Change in weight (gm./day)	Water intake (ml./day)
0·85 per cent D-leucine + 2·0 per cent DL-norleucine	12	40	39	−0·08	5·6
1·70 per cent D-leucine + 2·0 per cent DL-norleucine	5	39	44	0·95	7·8
0·85 per cent D-leucine + 2·0 per cent DL-norleucine	6	44	42	−0·30	6·3
2·20 per cent D-leucine + 2·0 per cent DL-norleucine	4	42	50	1·90	9·2
0·43 per cent L-leucine + 2·0 per cent DL-norleucine	7	50	51	0·15	8·7
0·43 per cent L-leucine	3	51	62	3·55	12·3
0·43 per cent L-leucine + 2·0 per cent DL-norleucine	6	62	58	−0·60	10·5
0·85 per cent L-leucine + 2·0 per cent DL-norleucine	3	58	69	3·65	14·3
0·43 per cent L-leucine + 2·0 per cent DL-norleucine	9	69	67	−0·20	10·7

Table 2. Effect of Norleucine and Related Compounds on the Growth of Weanling Rats Fed Diets Low in Leucine

Supplement to leucine-free diet	No. of rats	Initial weight (gm.)	Average weight gain (*W*) (gm.)	Average food intake (*F*) (gm.)	*W*/*F*
0·43 per cent L-leucine	4	57	16·0	71·5	0·22
0·43 per cent L-leucine + 1·0 per cent DL-norleucine	4	57	5·3	57·5	0·09
0·43 per cent L-leucine + 2·0 per cent DL-norleucine	4	57	2·0	62·0	0·03
0·43 per cent L-leucine + 0·99 per cent α-ketocaproic acid	4	57	8·5	56·0	0·15
0·43 per cent L-leucine + 0·78 per cent valeric acid	4	57	16·0	61·5	0·26
0·43 per cent L-leucine + 0·89 per cent caproic acid	4	57	19·3	73·5	0·26
0·43 per cent L-leucine + 1·00 per cent ε-aminocaproic acid	4	57	15·3	69·0	0·22

was without any demonstrable effect, it can be concluded that the deleterious effect manifested by norleucine is due to the intact molecule of the latter rather than to some of its catabolic products. Once the amino-acid undergoes oxidative decarboxylation it loses its growth-depressing effect. Since neither caproic nor ε-aminocaproic acids affected the growth of animals it is apparent that the amino- or oxo-group must be present at the α-carbon position in order to produce a growth depression in rats.

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Formation of Urea from Arginine in the Brain of the Living Rat

In a classical set of experiments, Bollman, Mann and Magath[1] demonstrated that "the production of urea in the body of the dog is entirely dependent on the presence of the liver, since urea formation ceases completely as soon as the liver is removed". Standard text-books of physiology and biochemistry have interpreted the above experiments as demonstrating that urea formation occurs exclusively in the liver. However, an alternative interpretation is possible, namely, that normal liver function is necessary for normal extra-hepatic metabolism, and such extra-hepatic metabolism may include urea synthesis. In fact, it has been shown that perfused brain requires factors elaborated or released by the liver to maintain normal metabolism[2].

Recently, our laboratory has demonstrated that the brain of the rat can metabolize proline to ornithine and arginine *in vivo*[3]. Because of the role of ornithine and arginine in the Krebs urea cycle[4], it was of interest to determine whether brain is capable of metabolizing arginine to urea. The present report describes such experiments.

In studying metabolism of the brain of the intact animal it is essential to control the variable of extra-cerebral metabolism if one wishes to draw valid conclusions about cerebral pathways. In order to control the variables (1) extra-cerebral metabolism and (2) alterations in the metabolism of the brain and in the permeability of the blood-brain barrier resulting from an intracisternal injection, the rats used in these experiments were injected as follows: rats 2 and 4 were injected intracisternally with 1·2 mgm. of uniformly labelled ^{14}C-L-arginine monohydrochloride (total activity 37 μc.); 5 min. after their intracisternal injections these same rats were injected in the exposed femoral vein with 1·2 mgm. of inert L-arginine monohydrochloride. Rats 1 and 3 (litter mates, respectively, of rats 2 and 4) were injected intracisternally with 1·2 mgm. of inert L-arginine monohydrochloride, and 5 min. later were injected in the exposed femoral vein with 1·2 mgm. of uniformly labelled ^{14}C-L-arginine monohydrochloride (total activity 37 μc.). If, by the use of the above method, one can show that rats 2 and 4, which received radioactive arginine intracisternally, have more radioactivity in urea isolated quantitatively from brain than rats 1 and 3, which received the same dose of radioactive arginine intravenously, then one may conclude that the brain itself has the ability to metabolize arginine to urea.

All injections were performed under light ether anæsthesia, and all rats were alert and walking within a few minutes after injections were finished. The animals were killed by decapitation 1 hr. after their injection of radioarginine, and their brains homogenized in trichloracetic acid. Following the addition of 8 mgm. of inert carrier urea, the homogenates were centrifuged and urea was isolated from

Table 1. RADIOACTIVITY IN BRAIN 1 HR. FOLLOWING INJECTION OF RADIOACTIVE ARGININE. ALL ACTIVITIES EXPRESSED AS COUNTS PER MIN.

	Rat 1	Rat 2	Rat 3	Rat 4
Brain (wet weight, gm.)	1·680	1·750	1·750	1·760
Total activity of injected arginine	27×10^6	27×10^6	27×10^6	27×10^6
Total activity of brain trichloracetic acid-soluble fraction	$2{\cdot}9\times10^4$	810×10^4	$4{\cdot}2\times10^4$	810×10^4
Activity/mgm. of dixanthydryl urea isolated from brain	43	1,500	46	1,710
Total activity in brain urea	2,600	89,000	2,700	101,000

the fractions soluble in trichloracetic acid by chromatography on 'Dowex 50' columns. Complete separation of urea from other components soluble in trichloracetic acid was achieved by making the dixanthydryl derivative of urea, which was then recrystallized to constant specific activity.

The results presented in Table 1 show that dixanthydryl urea isolated from rats 2 and 4 is 35–37 times more radioactive than that isolated from their litter-mate controls, rats 1 and 3. The results thus indicate that brain can form urea from arginine. Since it has previously been demonstrated that rat brain can synthesize arginine from proline[3], it appears that brain is capable of urea synthesis *in vivo*. However, the quantitative significance of this pathway as a method of ammonia detoxication in brain is not yet known, and the role of urea synthesis in the metabolism of the brain remains to be determined.

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Seasonal Variations in the Pituitary Gland of the Ferret

IT has been shown that the pars distalis of the pituitary gland of the cat, rabbit[1] and dog[2,3] contains cells which stain with carmine or acid fuchsin. These cells have been described as a second type of acidophile. They appear during the first pro-œstrous period in the young female cat, extend to occupy larger areas during œstrus and then partially regress[4].

Examination of the pituitary glands of female ferrets has shown that a similar type of carmine or fuchsin-staining cell is present, in addition to ordinary acidophiles which stain with orange *G* alone. After staining by the periodic acid – Schiff – orange *G* method[5] these 'carmine' cells appear deep reddish-orange in colour, in contrast to the yellow-orange of the acidophiles. This colour difference is due to a faint periodic acid – Schiff-positive reaction in the cytoplasm of the 'carmine' cells, combined with an affinity for orange *G*.

Pituitary glands of female ferrets killed at varying times of the year, and hence at varying phases of the breeding cycle, showed variations in the size and number of these cells. They were most numerous and widely distributed throughout the pars distalis in the glands of animals killed during early œstrus, and in four others which were anœstrous when killed in February, but which had been exposed to 16-hr. illumination each day for the three weeks before death. Bissonnette[6] showed that anœstrous ferrets reach full œstrus 38–64 days after exposure to extra light, and these four animals can almost certainly be considered to have been in an early pro-œstrous phase. In late œstrus, or early anœstrus, the cells were less conspicuous and reduced both in size and number; while in animals killed several months after the end of œstrus (November–January) the cells were either completely lacking or present only in small scattered groups. Vacuolation of the cells was seen in some, but not all, animals killed between August and December.

In the period immediately before œstrus, the cells are the largest of the various types in the pars distalis, and a Golgi zone similar to that found in basophiles is present in some of them. It seems probable that the increased size and number of these cells in the pro-œstrous period indicates that they are most active at this time; and this further suggests that they may be concerned with the initiation of œstrus.

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Measurements of Oxygen Tension in the Tissues of Mice cooled to 1° C.

Evans, Goodrich and Slaughter[1] demonstrated a decrease in the sensitivity to X-irradiation of the skin of new-born rats at body temperatures of about 0° C. Increased resistance to doses of X-rays which were lethal at normal body-temperatures has been demonstrated[2–3] at about 1° C. Weiss[4] has shown that there is increased resistance to radiation in the testes of mice subjected to whole-body X-irradiation at body-temperatures in the region of 1° C. It has been suggested by all of these authors that profound hypothermia acts by virtue of the anoxia which follows respiratory and cardiac arrest, and it is known that anoxia reduces sensitivity to X-irradiation[5].

We have recently attempted to measure oxygen tensions in the organs of mice while cooling, when

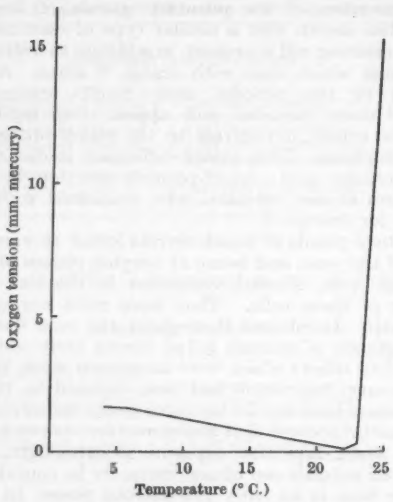

Fig. 1. Oxygen tensions recorded in the testis of a mouse resuscitated from 4° C.

cold, and also during resuscitation from the hypothermic state by the method of Goldsveig and Smith[6]. Gold electrodes (0·315 mm. diameter) insulated with 'Araldite' were inserted into spleen, testis and bone marrow and held in position by a stitch. A negative potential was applied (0·6 V. compared with a silver/silver chloride electrode inserted subcutaneously) and the resulting current was measured. Details of this oxygen–cathode technique for making quantitative measurements of oxygen tension will be described elsewhere (Cater, D. B., Silver, I. A., and Wilson, G. M., unpublished work).

Mice were cooled in sealed 1-lb. 'Kilner' jars immersed in ice, for 50 min. They were then removed from the jars, wetted with a detergent and surrounded by chipped ice, until their body temperatures measured per rectum fell to 0–1° C. After insertion of the electrodes, the ice was removed, the animals were warmed with a 60-W. electric light bulb, and given artificial respiration with a hand-pump.

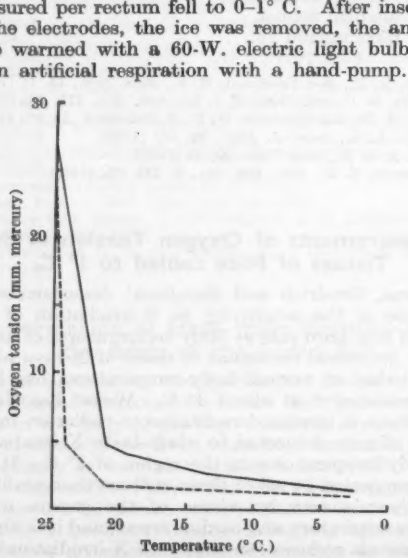

Fig. 2. Oxygen tensions recorded in the testis (———) and spleen (– – – –) of a mouse anæsthetized with urethane and then cooled by immersion in ice

The oxygen tensions recorded in the testis of a mouse, resuscitated from 4° C., are shown in Fig. 1. This experiment was discontinued when the animal showed signs of recovering consciousness.

It was not technically possible to make recordings of the oxygen tension in the organs of animals while they were being cooled down in a jar. However, mice were anæsthetized with subcutaneous injections of 25 per cent urethane in saline (0·25 ml./25-gm. mouse), and after the insertion of electrodes were cooled down in ice to 0–1° C. The recordings from the testis and spleen of such an animal are shown in Fig. 2. Respiration stopped in this mouse at 12° C.

The results showed that during cooling to the region of 0° C., the oxygen tension within the testis, spleen, bone-marrow and peritoneum fell reversibly to the order of 2 mm. mercury. This degree of hypoxia would be expected to afford considerable protection against radiation. The animals are protected from the effects of the hypoxia itself by their profound hypothermia. The possible applications of this to the radiotherapy of tumours will be discussed by one of us (L. W.) elsewhere.

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Target Organ Activation by Stress

It is a well-established fact that under stress conditions the organism calls for increased corticosteroid activity[1]. The response to such a call has been believed to occur solely by an increased corticosteroid production, that is, by increased pituitary-adrenocortical activity. Recent studies[2,3] have brought to light certain facts which suggest that the organism possesses an additional mechanism to meet the increased demand for corticosteroid activity. This mechanism functions by increasing the sensitivity of the target organs to corticosteroids.

The possibility of such a mechanism emerged during a study of the action of topically applied corticosteroids upon skin capillaries. It was noted that capillary resistance, measured by the negative pressure method, may increase under the local influence of corticosteroids in what appeared at first a haphazard manner. Further observation indicated that this increase in the response of the capillaries to corticosteroids is, in fact, a stress phenomenon.

An ointment containing 0·5 per cent Δ^2-hydrocortisone was applied on a circumscribed skin area of the flexor surface of the arm in human subjects or on the abdomen of dogs. The ointment base only was applied in the same manner on an adjacent control area. Five hours later the resistance of the

Table 1. Effect of Heavy Muscular Work (Gardening) on Capillary Resistance and Capillary Sensitivity of a 51-year-old Healthy Female Volunteer

A, Experimental day. Tests were applied at 7 a.m. (first line) and 2 p.m. (second line). Muscular exercise (arrow) was performed on the seventh day of experiment between the two testings. *B*, Capillary resistance (cm. mercury) in the skin area treated with cortisone. *C*, Capillary resistance in the control area. *D*, increase (+) or decrease (−) of the capillary resistance in cm. mercury in the cortisone-treated area as compared with the control area. An increase of more than 2 cm. was regarded as an indication of increased capillary sensitivity, that is, of capillary activation

A	*B*	*C*	*D*
5	11	12	− 1
	10	10	0
6	13	13	0
	12	11	+ 1
7	→ 13	12	+ 1
	24	16	+ 8
8	19	14	+ 5
	14	14	0
9	12	11	+ 1
	10	10	0
10	11	12	− 1
	12	10	+ 1

capillaries was measured in both areas (the value of capillary resistance is the least negative pressure expressed in cm. mercury which can produce one or more petechiae by rupturing the capillaries of the skin area tested). It was found that so long as the experimental subjects (two healthy volunteers and eight dogs) were leading a routine life and avoiding any extra stressful activity there was no difference between the resistance of the capillaries of the cortisone-treated and control skin areas amounting to more than ± 2 cm. mercury, the error of the method. The situation was markedly changed when stress was applied.

The human subjects did exhausting muscular work (swimming or gardening) or were exposed to ultra-violet light. On one subject the effect of delivering a strenuous class lecture was also studied. Dogs either ran in the treadmill or were irradiated by ultra-violet light or subjected to prolonged fasting. Following these various forms of stress the capillary resistance in skin area treated with cortisone was found to be significantly higher than in the control area in seven out of eight experiments in the human, and in eleven out of fourteen in the dogs. The differences showed wide individual variations amounting to 4–19 cm. mercury and were found to occur either within a few hours after stress or on the following day, and in most instances were demonstrable for several days. Two experiments, one in a human and one in a dog, exemplify in abbreviated form the phenomenon observed (Tables 1 and 2).

Table 2. Effect of a 25-day Fast (indicated between arrows). on Capillary Resistance and Capillary Sensitivity of an 18 kgm. Female Dog

For explanation see Table 1. Tests were made once daily between 1 and 2 p.m. During the period between the thirty-fifth and the forty-first days of the experiment no difference could be measured because capillary resistance in both areas was above the highest value measurable by the method (70 cm. mercury)

A	*B*	*C*	*D*
6	19	18	+ 1
8	19	20	− 1
10	19	19	0
12	20	18	+ 2
→ 14	21	20	+ 1
17	21	20	+ 1
19	24	19	+ 5
21	20	20	0
23	31	21	+ 10
25	27	20	+ 7
27	28	23	+ 5
29	30	25	+ 5
31	34	26	+ 8
33	30	30	0
35	> 70	> 70	
37	> 70	> 70	
→ 39	> 70	> 70	
41	> 70	> 70	
43	50	50	0
45	16	17	− 1
47	17	17	0
49	15	15	0

An attempt was then made to elucidate the mechanism by which capillaries are activated. There was no evidence that humoral factors play a part in this mechanism. Experiments on bilaterally sympathectomized dogs failed to demonstrate any capillary activation following stress. Activation was readily demonstrable in unilaterally sympathectomized dogs on the intact side but was entirely absent on the operated side. It appears, therefore, that activation, that is, increase in the sensitivity of the capillaries to corticosteroids, is intimately connected with the nervous system, specifically with the sympathetic nervous supply of the vessels.

The question whether or not other corticoid targets are activated by stress in a similar manner is open and does not appear to be as easily answerable as in the case of the capillaries. The present studies have in any event brought to light, through the specific example of the capillaries, a hitherto unknown ability of the organism to meet the demand induced by stress for increased corticosteroid activity in a manner other than by increased corticosteroid production.

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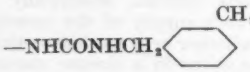
Preparation of an Electron-dense Antibody Conjugate

The fluorescent antibody conjugate technique recently developed by Coons[1] has already proved of great value in the study of biological problems. In this technique, a fluorescent substance is covalently linked to an antibody, and the antibody conjugate is allowed to react with cells or tissue containing its homologous antigen. The antigen is then detected and localized by observing the fluorescence in an optical microscope. For many reasons, it is desirable to extend this method to the sub-cellular level with the resolution attainable in the electron microscope. In order to achieve this purpose, however, it is necessary to confer sufficient electron density upon an antibody molecule, without inactivating it, to render it visible in the electron microscope.

We have prepared an electron-dense antibody conjugate by covalently coupling ferritin to the antibody. Ferritin is a crystalline protein of molecular weight 460,000 which is unique in that it contains as much as 23 per cent iron in ferric hydroxide–phosphate micelles[2]. Because of this

electron density, individual ferritin molecules may readily be seen in the electron microscope[3]; they exhibit a characteristic micellar arrangement at the corners of a roughly square lattice.

The coupling of ferritin to the antibody was carried out as follows. To 5 ml. of a 1·6 per cent solution of crystalline horse spleen ferritin[3] in sodium borate buffer, *p*H 9·5, Γ/2 0·1 at 0° C., was added 0·10 ml. of *m*-xylylene di*iso*cyanate (the generous gift of the Carwin Co., North Haven, Connecticut). After ¾ hr. stirring at 0° C., the mixture was centrifuged, the supernatant solution was removed from the unreacted di*iso*cyanate, and was allowed to stand at 0° C. for an additional hour to allow any dissolved di*iso*cyanate to react. At this point, roughly half the free amino-groups of ferritin had reacted, presumably converted to



groups. This solution was then added to 5 ml. of a 1·6 per cent solution of rabbit γ-globulin (containing specific antibody), in the above-mentioned borate buffer containing, in addition, 1 *M* sodium chloride. After two days stirring at 6° C., the mixture was dialysed against 0·1 *M* ammonium carbonate to destroy any unreacted *iso*cyanate groups, and then was dialysed against phosphate buffer, *p*H 7·5, Γ/2 0·1. A small amount of precipitated protein was removed by centrifugation to give a clear stable solution.

To demonstrate that (*a*) the ferritin was conjugated to antibody, and (*b*) the ferritin–antibody conjugate was still capable of reacting specifically with its homologous antigen, the following experiments were performed. Two preparations of rabbit γ-globulin were available, one containing about 25 per cent anti-bovine serum albumin (BSA)Ab, the other about 20 per cent anti-bovine ribonuclease (RNase)Ab. Ferritin was treated with both by the procedure described above, giving preparations designated Fer-anti-BSA and Fer-anti-RNase, respectively. Two mixtures were then made, containing: (1) 2 mgm./ml. of Fer-anti-BSA and 1·5 mgm./ml. of unreacted anti-RNase γ-globulin; and (2) 3 mgm./ml. of Fer-anti-RNase and 1 mgm./ml. of unreacted anti-BSA γ-globulin, each in phosphate buffer, *p*H 7·5, Γ/2 0·1. 2-ml. portions of each mixture were then added to various dilutions of either RNase or BSA, and the precipitates formed after 24 hr. at 4° C. were washed and analysed for iron by the *o*-phenanthroline procedure, and for nitrogen by Nessler analysis.

Consider the results in the three upper sections (*A*, *B* and *C*) of Table 1. These clearly demonstrate that the presence of Fer-anti-BSA had no effect on the precipitation of RNase by anti-RNase, and therefore that the iron brought down in the RNase-(Fer-anti-RNase) precipitate must have been bound to specifically active antibody. Consistent with this conclusion is the essential independence of the iron/nitrogen ratio from the antigen/antibody ratio in the RNase-(Fer-anti-RNase) precipitates. Furthermore, if we assume that each Fer–Ab conjugate contains one ferritin and one antibody molecule, it follows from the iron/nitrogen ratios of 2·0 in our ferritin sample itself, and of 0·8 in the RNase-(Fer-anti-RNase) precipitates, that at least one-third of the antibody molecules were conjugated to ferritin. A larger fraction of antibody molecules could probably be coupled by using a larger mole ratio of ferritin to γ-globulin in the coupling reaction.

Table 1. Ferritin–Antibody Coupling Experiments

	Antigen (mgm.)	Antibody-containing γ-globulin	Antigen–antibody precipitates N (mgm.)	Fe (mgm.)	Fe/N
A	RNase, 0·125	anti-RNase	0·044	0	0
	0·063		0·100	0	0
	0·031		0·083	0	0
	0·016		0·046	0	0
B	RNase, 0·125	anti-RNase / Fer-anti-BSA	0·039	0·008	0·21
	0·063		0·097	0·009	0·09
	0·031		0·090	0·008	0·09
	0·016		0·051	0·007	0·14
C	RNase, 0·125	anti-BSA / Fer-anti-RNase	0·009	0·015	—
	0·063		0·043	0·048	1·1
	0·031		0·149	0·119	0·80
	0·016		0·084	0·068	0·81
	0·008		0·049	0·046	0·94
D	BSA, 0·250	anti-BSA	0·058	0	0
	0·125		0·113	0	0
	0·063		0·089	0	0
	0·031		0·046	0	0
E	BSA, 0·250	anti-BSA / Fer-anti-RNase	0·008	0·003	—
	0·125		0·134	0·034	0·25
	0·063		0·125	0·064	0·51
	0·031		0·095	0·064	0·67
	0·016		0·061	0·057	0·93
F	BSA, 0·250	anti-RNase / Fer-anti-BSA	0·009	0·005	—
	0·125		0·067	0·049	0·73
	0·063		0·133	0·098	0·74
	0·031		0·078	0·052	0·67

In BSA-anti-BSA precipitations from solutions containing Fer-anti-RNase (section *E*, Table 1), some ferritin was brought down non-specifically. However, the iron/nitrogen ratio varied markedly with the antigen/antibody ratio in the precipitate. In particular, the iron/nitrogen ratio near the antigen-antibody equivalence zone was considerably smaller than the ratio in the specific BSA-(Fer-anti-BSA) precipitate (section *F*, Table 1). These results, therefore, while somewhat obscured by the non-specific precipitation, are consistent with the conclusions derived from the ribonuclease system.

The question of the specificity with which a Fer–Ab conjugate reacts with its homologous antigen is vital to the use of such conjugates in electron microscopic studies of cell and tissue sections. This question can only be answered at the electron microscopic level, however, and not by macroscopic antigen–antibody precipitation experiments. The specific behaviour of the RNase system, and the non-specific complications in the BSA system, are therefore not directly pertinent to this question.

Preliminary electron microscopic studies, in collaboration with Prof. William R. Adams, have indicated that in preparations of Fer–Ab conjugates, the characteristic micellar structure observed with ferritin itself is unaffected. These and further studies in progress, dealing with the application of the Fer–Ab conjugates to problems at the electron microscopic level, will be reported in detail elsewhere.

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Inhibition of Electron Transport and the Swelling of Isolated Mitochondria

THE swelling of isolated rat-liver mitochondria appears under certain conditions to be dependent on their respiration[1]. This view is based on the following observations. (*a*) Mitochondria 'aged' by storage for one day in 0·44 *M* sucrose at 0° C. did not swell in presence of phosphate or thyroxine unless an oxidizable substrate was added as well. (*b*) With succinate as the substrate, malonate inhibited the swelling and amytal did not; whereas with β-hydroxybutyrate, amytal inhibited and malonate did not. (Amytal prevents oxidation of substrates with diphosphopyridine nucleotide-linked dehydrogenases, but has no effect on that of succinate[2].) (*c*) Cyanide inhibited swelling induced by phosphate, but not when electron transport was restored by addition of ferricyanide as terminal acceptor[3]. (*d*) Antimycin *A* inhibited swelling in presence of phosphate with various substrates[1,3]. The effects of amytal and antimycin on swelling have now been investigated further.

The experimental conditions have been described previously[1]. Antimycin in concentrations known to inhibit many mitochondrial oxidations[4] (3–50 mμgm./ml.) had only a small effect on the swelling induced by 10 m*M* phosphate or 30 μ*M* L-thyroxine with fresh mitochondria. In contrast, with aged mitochondria in the presence of 1 m*M* D-β-hydroxybutyrate, 5–8 mμgm. antimycin/ml. completely arrested the swelling induced by phosphate or thyroxine. However, when the β-hydroxybutyrate was replaced by L-glutamate, L-malate, L-proline or succinate (all 1 m*M*) the inhibition of phosphate-induced swelling was much less, and was only partial even with 50 mμgm./ml. (Fig. 1). A selective effect was observed with amytal as well. Unlike antimycin, amytal[2] (1·8 m*M*) completely arrested the swelling of fresh mitochondria induced by phosphate or thyroxine. With aged mitochondria, however, amytal resembled antimycin (Fig. 1), in that when β-hydroxybutyrate was present, a given inhibition was produced by a lower concentration of the barbiturate than that which was required with glutamate (Fig. 2).

Fresh mitochondria have been shown to contain substrates, for example, citrate[5] and precursors of glutamate[6], and to have a significant endogenous respiration[7]. It is therefore reasonable to suppose that the swelling of fresh particles is supported by oxidation

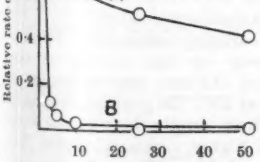

Fig. 1

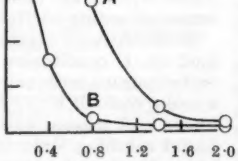

Fig. 2

Figs. 1 and 2. Effects of antimycin *A* (Fig. 1) or amytal (Fig. 2) on the swelling induced by phosphate of aged mitochondria with 1 m*M* D-β-hydroxybutyrate or L-glutamate as substrates. The ordinates represent the steady rate of swelling in the presence of inhibitor relative to the maximal rate in an uninhibited control. Each cell contained 0·3 *M* sucrose, 25 m*M* *tris* chloride buffer, pH 7·5, and 10 m*M* phosphate. 20° C. Curve *A*, L-glutamate; curve *B*, β-hydroxybutyrate

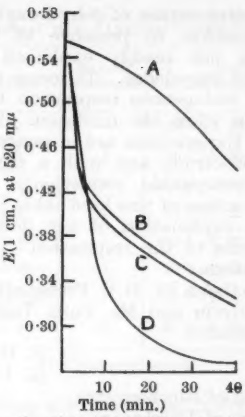

Fig. 3. Effects of antimycin *A* (20 mμgm./ml.) on swelling induced by phosphate of fresh mitochondria with and without D-β-hydroxybutyrate. Experimental conditions as for Figs. 1 and 2. Curve *A*, phosphate, β-hydroxybutyrate + antimycin; curve *B*, phosphate + antimycin; curve *C*, phosphate; curve *D*, phosphate + β-hydroxybutyrate

of intrinsic material. We have seen that antimycin in the concentrations tested had little effect on the swelling of fresh mitochondria in absence of added substrate, whereas it was profoundly inhibitory with aged particles in presence of β-hydroxybutyrate. If this substrate were added to fresh mitochondria, it might be expected that antimycin would remain ineffective, swelling still being supported by the endogenous respiration, which is not sensitive to antimycin. Surprisingly, however, 5–50 mμgm. of antimycin/ml. strongly inhibited the phosphate-induced swelling of fresh mitochondria in the presence of β-hydroxybutyrate (Fig. 3). This suppression of the phosphate swelling was not obtained when β-hydroxybutyrate was replaced by malate; glutamate gave a slight effect. With ferricyanide present as terminal electron acceptor, antimycin had a similar action, that is, the phosphate-induced swelling, which was little affected by the antibiotic, was almost completely inhibited in the presence of β-hydroxybutyrate[3].

The action of the combination of β-hydroxybutyrate and antimycin was not restricted to fresh mitochondria, for with aged particles in which swelling due to phosphate was supported by glutamate, the further addition of DL-β-hydroxybutyrate together with antimycin completely checked the increase in volume, whereas antimycin alone caused an inhibition of only about 50 per cent.

In some respects oligomycin, which inhibits the phosphorylation system and thereby considerably reduces respiration in coupled systems[8], has an action on mitochondrial swelling like that of antimycin. Oligomycin (0·4 μgm./ml.) had a very small effect on the phosphate-induced swelling of fresh mitochondria, but when DL-β-hydroxybutyrate was present as well, almost complete inhibition resulted. In contrast to that of antimycin, this effect was counteracted by 2 : 4-dinitrophenol[3].

To summarize, it has been shown that antimycin and amytal caused an inhibition of mitochondrial swelling which depended on the substrate present; maximal sensitivity to these agents was observed when β-hydroxybutyrate was present, and minimal effects with no added substrate, that is, when the mitochondria were presumably dependent on their endogenous respiration. Other substrates showed behaviour between these extremes.

The protective action of β-hydroxybutyrate with fresh mitochondria in presence of antimycin or oligomycin is not readily explained in the light of our present knowledge. It seems to indicate an inhibition of endogenous respiration by β-hydroxybutyrate even when the oxidation of the latter is suppressed. Experiments are in progress, both with the oxygen electrode and with a new method for measuring ferricyanide reduction[3], to determine whether interaction of this kind takes place. If none is found, an explanation of our findings must be sought in terms of the respiration – swelling transducer mechanism.

We wish to thank Dr. B. C. Pressman for a generous gift of oligomycin and Mr. Colin Taylor for expert technical assistance.

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Methanol-soluble Carbohydrate Complexes

We have found that carbohydrates of low molecular weight are generally highly soluble in concentrated solutions of anhydrous calcium chloride in cold absolute methanol. After some time, crystalline methanol-solvated complexes of calcium chloride and carbohydrate are deposited. Such solutions and methanol-solvated complexes have not been reported before.

Lactose is the carbohydrate we have studied most thoroughly. A highly concentrated solution is prepared, for example, by mixing at 40° C. lactose, calcium chloride and methanol in a molar ratio such as 1 : 3 : 24. Paper chromatographic analysis of the aged solution shows that more than 97 per cent of the lactose remains unchanged and that a minor constituent has formed to the extent of less than 3 per cent. The specific rotations have been measured in water; $[\alpha]_i$ refers to the value extrapolated to the instant of mixing with water, and $[\alpha]_f$ to the later equilibrium value; the rotational effects of the calcium chloride and methanol are negligible under the conditions of measurement. That the α-form of lactose exists in higher proportion in methanolic calcium chloride solution than in water is shown by the ratio of $[\alpha]_i$ to $[\alpha]_f$, which equals 1·30. However, since $[\alpha]_f$ of the lactose in the methanolic solution is 43·5, while $[\alpha]_f$ of the lactose used as starting material is 55·3, the minor constituent which was found in the chromatographic analysis must have a very low or negative rotation.

From the highly concentrated methanolic solution, heavy crystallization occurs after months, or, when it is seeded and shaken, after several days or weeks. The crystals are separated by prolonged centrifugal filtration in a closed system, since they cannot be washed without change in composition. The crystals are first dried at 60° C. to evaporate the methanol, and then at 105° C. If heated first at 105° C., they decompose. Analysis shows that the crystals consist of 1 mole of lactose, 1 mole of calcium chloride, and 4 moles of methanol. Polarimetry shows that nearly all the lactose is present in its β-form.

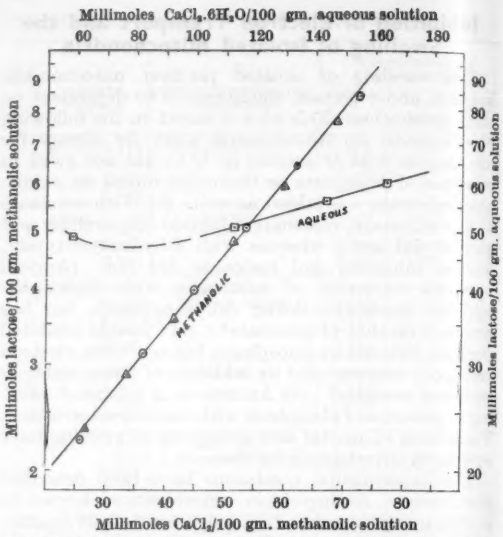

Fig. 1. ▲, ○, Solubility of lactose in methanolic calcium chloride, from two similar sets of experiments; ⊡, solubility of lactose in aqueous calcium chloride, according to Herrington (ref. 1).

The dilution of the concentrated lactose solution with methanol causes lactose to crystallize. The ratio of $[\alpha]_i$ to $[\alpha]_f$ for these crystals equals 1·28; the final rotation $[\alpha]_f$ is 54·7. While this ratio is the same as exists in solution, these crystals do not contain the minor constituent which affects the final rotation in solution. Within the range investigated, the logarithm of the solubility of this crystallized lactose in methanol is a linear function of the calcium chloride concentration (Fig. 1). Herrington[1] determined the solubilities of lactose in aqueous calcium chloride solutions. If one plots (Fig. 1) the results of his four tests in which lactose was the solid phase, the logarithm of the lactose concentration turns out to be another straight line for the highest of three points; the lowest point is off the line.

When about 6 moles of water are added to the highly viscous methanolic lactose solution for each mole of dissolved calcium chloride, the maximum amount of a complex consisting of (lactose. $CaCl_2.7H_2O$) gradually crystallizes over a period of a few days. Adding either more water or less lowers the yield. The lactose in these crystals is the α-form. These crystals are identical with those deposited from water according to Herrington's procedure[1].

Solubilities of several carbohydrates were determined in a qualitative way by the mixing of the carbohydrate with calcium chloride and methanol in a molar ratio of 1 : 3 : 24 at 28°. In general, monoses, bioses and trioses, and their simple derivatives were found soluble; only the higher polysaccharides (for example, dextrin) are insoluble. The following are soluble: the sugars D-glucose, D-galactose, L-sorbose, D-fructose, sucrose, maltose, melezitose and raffinose, and the sugar derivatives α-methyl-D-glucoside, D-gluconolactone, sorbitol, lactobionolactone, and lactositol.

Some of the monoses, for example, glucose and fructose, crystallize very rapidly as methanol-solvated

calcium chloride complexes. The fructose solution is of special interest in that soon after it begins to crystallize it becomes a translucent homogeneous rigid gel which fills the entire volume of the solution.

Methanolic calcium chloride might serve as a solvent for sugar reactions and for fractionating sugar mixtures by extraction or crystallization.

A fuller report is to be published elsewhere.

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Effects of Vanadium upon Liver Coenzyme *A* in Rats

THE addition of vanadium to liver sections of normal animals can prevent the synthesis of cholesterol from both mevalonic acid and acetate[1]. Diets containing vanadyl sulphate do not, however, alter the cholesterol-levels in the serum or liver after being administered for six weeks to rabbits fed on high-cholesterol diets, though they considerably reduce the cholesterol concentration in the aorta and appreciably lower the capacity of the liver to synthesize cholesterol[1,2].

On these grounds, a study was carried out aimed at assessing the effects of vanadium upon the liver coenzyme *A*—for it is an established fact that the synthesis of cholesterol from acetate is promoted by the activation of acetate by coenzyme *A*.

The first experiments were devoted to investigating the effects of vanadium upon the serum and liver cholesterol contents in (1) normal rats, (2) rats that had been kept on a high-cholesterol diet and (3) rats that had been administered intravenous injections of 'Triton' *WR* 1339 (arylalchylic phenol polyether) a surface-active substance that can enhance the cholesterol-synthesizing capacity of the liver[3]. Other experiments were devoted to assessing the effects of vanadium upon the coenzyme *A* of the liver.

The test were performed on white rats of our breeding, average weight 90–120 gm., fed *ad libitum* for 7 days before the experiment on a standard diet (diet *A*) of 21 per cent casein, 7 per cent olive oil, 4 per cent salt mixture IV, 5 per cent vitaminized lactose and 63 per cent sugar.

The hyperlipæmia from 'Triton' *WR* 1339 was determined by injecting the surface-active substance intravenously. Vanadium, in the form of an aqueous solution of sodium metavanadate ($NaVO_3.H_2O$), was simultaneously administered by intraperitoneal injection. The animals were killed by decapitation 18 hr. after the injection, and their sera and livers were analysed for total cholesterol by the Sperry–Webb[4] and Abell[5] methods, respectively.

Table 1. EFFECTS OF VANADIUM UPON THE CHOLESTEROL CONTENTS IN THE LIVER AND SERUM OF RATS UNDER VARYING EXPERIMENTAL CONDITIONS (AVERAGE VALUES ± S.E.)

No. of animals	Diet	Triton (mgm./kgm.)	Vanadium	Total cholesterol (mgm./100 gm. or 100 ml.) Serum	Liver
10	*A*	—	—	71·4 ± 4·33	204·5 ± 9·78
12	*A*	—	5 mgm./kgm. i.p.*	67·6 ± 3·48	182·9 ± 7·06
12	*A*	200	—	264·9 ± 12·04	1,083·8 ± 38·75
13	*A*	200	5 mgm./kgm. i.p.	197·2 ± 11·81	741·8 ± 21·14
15	*B*	—	—	80·6 ± 5·27	725·3 ± 24·33
15	*B*	—	0·01 per cent in diet	74·9 ± 6·10	640·5 ± 19·84
15	*B*	—	0·05 per cent in diet	76·6 ± 6·32	597·4 ± 15·45

* i.p., intraperitoneal.

Table 2. EFFECTS OF VANADIUM ON THE COENZYME *A* IN THE LIVER OF NORMAL RATS (AVERAGE VALUE ± S.E.)

No. of animals	Vanadium	Days of treatment	Coenzyme *A* u./gm. liver	u./100 mgm. protein
12	—	—	138·1 ± 3·75	103·5 ± 3·29
15	5 mgm./kgm. i.p.*	1	135·7 ± 3·49	94·9 ± 3·12
15	5 mgm./kgm. i.p.	6	101·4 ± 2·64	81·1 ± 2·40
15	10 mgm./kgm. i.p.	1	83·0 ± 2·12	68·5 ± 2·18
12	0·01 per cent in diet	7	124·5 ± 3·60	98·3 ± 2·78
12	0·01 per cent in diet	14	97·6 ± 2·35	84·7 ± 2·11
12	0·05 per cent in diet	7	91·2 ± 2·07	76·0 ± 2·42
12	0·05 per cent in diet	14	68·5 ± 2·15	59·4 ± 1·29

* i.p., intraperitoneal.

The results are shown in Table 1, together with those concerning the effects of vanadium upon the cholesterol content of the serum and liver of normal subjects. In the same table the results obtained by administering vanadium for 7 days with a diet *A* containing an extra 1 per cent cholesterol (diet *B*) are also tabulated.

The effects on the coenzyme contents of the liver of vanadium administration, either parenterally or incorporated in our diet *A*, are tabulated in Table 2. The coenzyme content was determined by the Kaplan and Lipmann method[6] on two-thirds of each liver, the remainder being used for protein determination by the Folin method as amended by Lowry[7].

From Table 1 it can be seen that no significant changes occur in the cholesterol-levels in the liver and serum of normal animals 18 hr. after the vanadium injection. Now, since the same vanadium dose appears to lower to a fair extent the cholesterol-levels in the animals treated with 'Triton' the assumption that the metal exerts its effect only when cholesterol synthesis has been abnormally enhanced appears to be justified.

In animals kept on a high-cholesterol diet, the amount of cholesterol in the liver is reduced, though only to a moderate extent, even when only 0·01 per cent vanadium is added to the food. (The effect remains more or less unchanged as the vanadium percentage is increased.)

The effect of vanadium injected intraperitoneally on the coenzyme *A* of the liver becomes evident 24 hr. after a single administration of 10 mgm./kgm., or 6 days after a 5 mgm./kgm. daily dose.

When administered in the diet at a concentration of 0·01 per cent over 7 days, the metal does not seem to exert any significant action, the effects becoming apparent after a 14-day treatment. At a concentration of 0·05 per cent in the diet, a reduction in the coenzyme *A* of the liver is observable after 7 days; after a 14-day treatment it becomes considerable and may be as much as 40 per cent less than normal.

Our investigations have not yet detected the mechanism of the inhibiting action of vanadium upon the coenzyme *A*. Other workers[8] have suggested that the metabolism of sulphurated amino-acids would be affected by vanadium. If so, considering the important role of the thiolic cell components in

biosynthesis of the coenzyme, a shortage might be postulated of the sulphydryl groups, notably cysteine, that are needed for the formation of pantotheine.

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Separation of Mono-*n*-butyl Phosphate and Di-*n*-butyl Phosphate by means of Ion Exclusion

Many different methods have been applied by several authors to the separation of mono- and di-*n*-butyl phosphate. Kumler and Eiler[1], who were interested in obtaining pure samples for the determination of their dissociation constants, used fractional precipitation of barium salts. Extraction by organic solvents (*n*-amylic alcohol or dibutylic ether) was applied with a good yield by Stewart and Crandall[2]; di-*n*-butyl phosphate is preferentially extracted into the organic phase. With the aim of obtaining mono- and di-*n*-butyl phosphate labelled with phosphorus-32, after the isotopic transfer of phosphorus-32 from $H_3{}^{32}PO_4$ to the esters, the exchange resin 'Dowex 2 *X*–8', 100–200 mesh, chloride form, was used by Higgins and Baldwin[3] to separate the three components from each other. Thus the mixture at *p*H 9 was fixed at the top of the resin bed; *ortho*-phosphoric acid, mono- and di-*n*-butyl phosphate were consecutively obtained by eluting with 0·05 *M* hydrochloric acid and with 0·5 *M* potassium chloride.

Separations of the same type were also carried out in our laboratory with satisfactory results, by using 'Amberlite *IRA* 400', carbonate form, and 0·1 *M* sodium carbonate and 0·1 *M* sodium chloride as selective eluants.

To investigate another way of separation, some ion exclusion experiments were carried out by us. This method needs no regeneration of the resin, which acts with a different mechanism from that of common ionic exchange. Many factors are reported to determine the limitations of ion exclusion[4]; among them are: rate of flow of solution through the column; volume of feed and concentration ratio of the two components; operating temperature; size of resin particles and percentage of cross-linkage; degree of dissociation of compounds to be separated. In principle, separation through ion exclusion is effective when compounds involved possess different degrees of dissociation, because one component acts as an 'ionic substance' in respect to the other 'non-ionic'.

Even though mono- and di-*n*-butyl phosphate have nearly the same dissociation constants[1] (for mono-*n*-butyl phosphate $K' = 1{\cdot}29 \times 10^{-2}$, $K'' = 1{\cdot}40 \times 10^{-7}$ and for di-*n*-butyl phosphate $K = 1{\cdot}90 \times 10^{-2}$) a fairly good separation was obtained by operating in the conditions reported below. A bed of 50 c.c. of 'Dowex 50 *X*–8', 50–100 mesh, hydrogen form, having a cross-section of 1·1 cm.², was fed with 9 ml. of a solution containing 0·071 m.moles/ml. of mono-*n*-butyl phosphate and 0·037 m.moles/ml. of di-*n*-butyl phosphate at a flow-rate of 6·25 ml./hr. cm.². While eluting at room temperature (18° C.), with distilled water at the same rate of flow, fractions of 2·2 ml. were automatically collected. Results are shown in Fig. 1, where mono-*n*-butyl phosphate is shown appearing after 18 ml. of effluent and di-*n*-butyl phosphate after 38 ml., as was expected on a theoretical base. Furthermore, it should be pointed out that mono-*n*-butyl phosphate, in spite of its slightly lower dissociation constant, is eluted first. The yield of separation is 95·5 per cent in pure mono- and 89 per cent in pure di-*n*-butyl phosphate, since in one fraction alone the two components were both present. Rate of flow can be raised to 9 ml./hr. cm.² and feed concentration varied at about ±10 per cent without influencing the separation yield, which is lowered only when the amount of resin is reduced by 20 per cent.

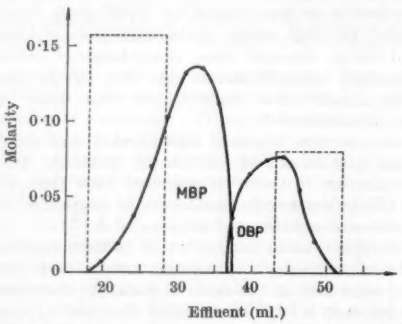

Fig. 1. Elution curves for mono- and di-*n*-butyl phosphate. Dashed lines indicate the theoretical shape of the separation

To prepare significant amounts of pure compounds, seven semi-continuous cycles were carried out, on 650 c.c. of resin, 5·2 cm.² cross-section. Each cycle employed 100 ml. of feed solution containing 0·061 m.moles/ml. of mono-*n*-butyl phosphate and 0·028 m.moles/ml. of di-*n*-butyl phosphate, at a rate of flow of 9 ml./hr. cm.².

All the experiments described here were performed on solutions of mono- and di-*n*-butyl phosphate obtained synthetically from phosphorus pentoxide and butanol, and brought to suitable concentrations by dilution with water.

Analyses of fractions were carried out by potentiometric titration with 0·1 or 0·01 *N* sodium hydroxide; in some cases a purity control by paper chromatography was effected[5].

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Structure of Dihydrodiphosphopyridine Nucleotide

WEBER[1] has recently reported on the intramolecular transfer of excitation energy in dihydrodiphosphopyridine nucleotide. In addition to the strong band having its maximum at 340 mμ, the excitation spectrum of the 462 mμ fluorescence also shows a maximum at 260 mμ, a region where the absorption is due to the purine part of the molecule.

Kaplan *et al.*[2] have identified an isomer of diphosphopyridine nucleotide which comprises 10–15 per cent of a highly purified commercial preparation of diphosphopyridine nucleotide. The isomer was characterized by optical rotation studies as the α-glycoside of nicotinamide riboside as distinct from the biologically active β-isomer of diphosphopyridine nucleotide. This assignment of configuration about the C_1 of ribose was substantiated by the synthetic preparation of nicotinamide mononucleotide[3] to give a mixture of the β- and α-anomers in a 4 : 1 ratio.

The α-isomer of diphosphopyridine nucleotide was reduced chemically with sodium dithionite[4] and the product was examined spectrofluorometrically. Unlike the β-isomer, the excitation spectrum of the 462 mμ fluorescence of α-dihydrodiphosphopyridine nucleotide shows a strong band only when activated at 340 mμ and not when irradiated at 260 mμ, indicating that excitation energy transfer is not as efficient with the α-isomer as was observed with the β-isomer. The 462 mμ fluorescence resulting from 260 mμ and 340 mμ excitation of α- and β-dihydrodiphosphopyridine nucleotide is shown in Fig. 1.

Further evidence for the differences in interaction in the α- and β-isomers of dihydrodiphosphopyridine nucleotide may be found in their spectrophotometric behaviour in water and propylene glycol. Weber[1] reported that the ratio E_{260}/E_{340} is 2·85 for the intact β-isomer of dihydrophosphopyridine nucleotide, whereas this ratio becomes 3·4 in propylene glycol. A similar examination of the reduced α-isomer reveals that the E_{260}/E_{340} ratio is the same in water as in the propylene glycol.

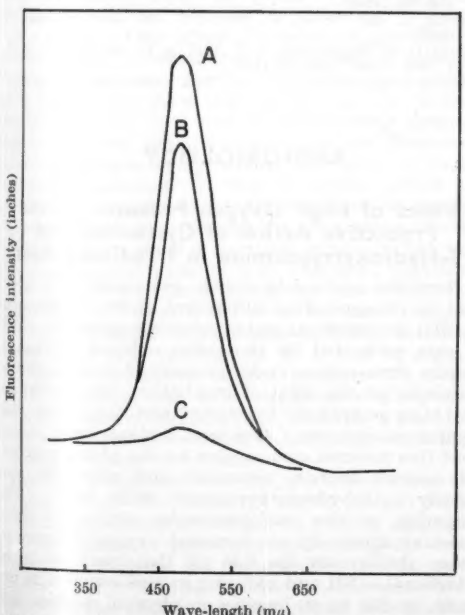

Fig. 1. Fluorescence spectra of α- and β-dihydrodiphosphopyridine nucleotide. Curve *A*, α- and β-forms excited at 340 mμ; curve *B*, β-form excited at 260 mμ; curve *C*, α-form excited at 260 mμ. These values are relative since the intensities of the exciting light have not been determined

Studies similar to those described for the α-isomer of dihydrodiphosphopyridine nucleotide have been carried out with deamino-dihydrodiphosphopyridine nucleotide (the hypoxanthine analogue[5]) and with the 3-acetylpyridine analogue[6]. The latter analogue clearly showed the transfer of excitation energy since excitation at 260 mμ gives a 478-mμ fluorescence[7] which is also elicited by excitation at 365 mμ. The absorption spectrum of this analogue behaved much like β-dihydrodiphosphopyridine nucleotide in water and propylene glycol. The deamino-dihydrodiphosphopyridine nucleotide, however, did not show properties of energy transfer, since excitation of the purine ring did not result in fluorescence of the dihydropyridine nucleus.

In accordance with the postulate by Weber[1] that the adenine and the dihydropyridine rings form a complex in β-dihydrodiphosphopyridine nucleotide, it appears that such complex formation cannot occur in the α-isomer.

Further studies on the structural conformation of diphosphopyridine nucleotide and dihydrodiphosphopyridine nucleotide are being examined in this laboratory through the use of pyridine coenzyme analogues.

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A Possible Negative Feedback Phenomenon controlling Formation of Alkaline Phosphomonoesterase in *Escherichia coli*

WE have already reported[1] that in a phosphate-deficient medium the amount of ribonucleic acid in *E. coli* decreased remarkably, while there was an appreciable increase of deoxyribonucleic acid, protein and viable cell numbers. At the same time we also studied activities of some enzymes presumed to be related to the degradation of ribonucleic acid, for example, ribonuclease phosphodiesterase and phosphomonoesterase, and found a marked increase in that of phosphomonoesterase. This communication is concerned with this formation of phosphomonoesterase in *E. coli*.

The strain used was *E. coli B* and the medium contained per litre: 3·0 gm. NaCl, 0·25 gm.

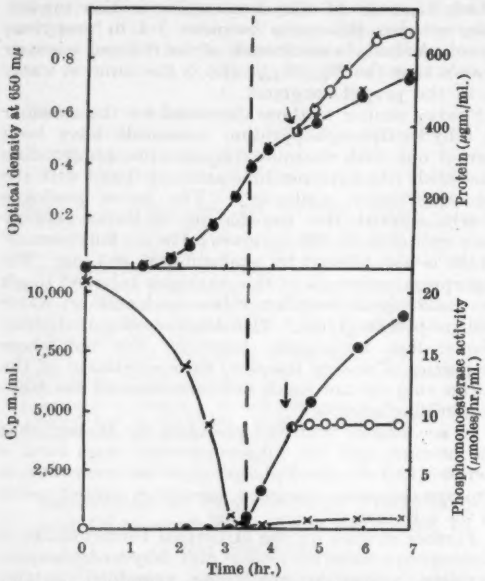

Fig. 1. Relation between the formation of phosphomonoesterase and phosphate content in the medium. Cells were suspended in a medium containing phosphorus-32 at 10 μgm. phosphorus/ml. and incubated at 37° C. with shaking. At the time indicated aliquot samples were taken to assay phosphomonoesterase activity and protein content (see text). At the time indicated by arrows, the culture was divided into two portions, to one of which (○—○) was added phosphate (100 μgm./ml.) and to the other (●—●) water. Radioactivity of the medium was obtained from that of the supernatant fraction after centrifugation (10,000*g*; 5 min.). The broken line indicates the time when the phosphate in the medium was exhausted. ●, ○, Optical density; ×, protein concentration

$MgSO_4.7H_2O$, 0·01 gm. $CaCl_2$, 2·0 gm. sodium lactate, 10·0 gm. Difco 'Bactopeptone' and 12 gm. *tris*-(hydroxymethyl)-aminomethane. The *p*H was 7·4. This medium contained 16 μgm. phosphorus/ml. Phosphomonoesterase activity was measured by the method of Bessey *et al.*[2], using *p*-nitrophenyl phosphate as the substrate, glycine–sodium hydroxide buffer (*p*H 9·5) and magnesium sulphate. Activity of phosphomonoesterase was expressed as μmoles of *p*-nitrophenyl phosphate hydrolysed/hr./ml. of toluene-lysed cell suspension[3]. Protein was estimated by the method of Lowry *et al.*[4] using bovine serum albumin as standard.

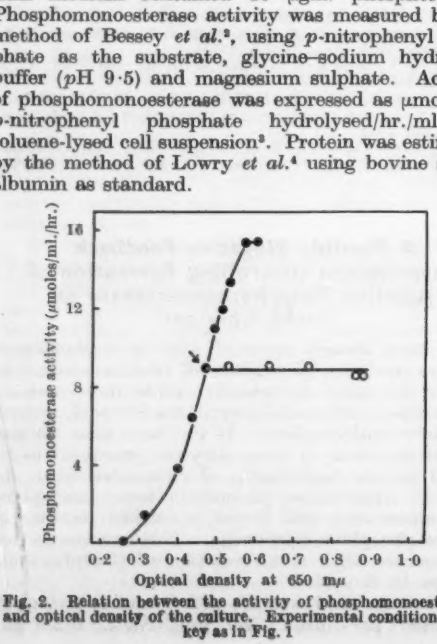

Fig. 2. Relation between the activity of phosphomonoesterase and optical density of the culture. Experimental conditions and key as in Fig. 1

As illustrated in Fig. 1, cells began to form phosphomonoesterase when the phosphate in the medium was exhausted. Thereafter, a linear increase of activity was observed for 2–3 hr. and the amount of cell protein/ml. increased linearly, although at a low rate. Fig. 2 shows the relation between the increase of optical density and that of the phosphomonoesterase activity. As shown in Fig. 2 this increment of protein was directly proportional to that of phosphomonoesterase activity, except in the early and in the final phases of the phosphate-deficient stage. The formation of phosphomonoesterase was stopped immediately by the addition of phosphate to the medium (final concentration: 100 μgm. phosphorus/ml.), though the amount of protein increased at a high rate (Figs. 1 and 2). The appearance of phosphomonoesterase activity was not observed in the phosphate-rich medium. Apparently phosphate inhibits the action of phosphomonoesterase.

In view of the above results, it seems to us that the formation of phosphomonoesterase is an important factor in the regulation system of the phosphate economy in *E. coli*. A negative feedback mechanism[5] is possible, with phosphate as a repressor, although the activation of phosphomonoesterase cannot be ruled out. Further details will be published elsewhere.

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RADIOBIOLOGY

Effect of High Oxygen Pressure on the Protective Action of Cystamine and 5-Hydroxytryptamine in Irradiated Rats

Both the oxidized disulphide, cystamine (—S—S—) and its corresponding sulphydryl amine, cysteamine (—SH) are excellent radioprotective agents *in vivo*[1]. If rats, protected by the latter reduced substance, respire pure oxygen under pressure (4–5 atmospheres absolute) at the time of irradiation, the protective action is completely reversed[2,3] and this result also applies to cysteine. Our own findings have shown that this reversal also applies to the pharmacologically active amines, histamine and adrenalin, and largely to 5-hydroxytryptamine (*vide infra*). The lessening of the radioprotective action of these reducing agents by an increased oxygen pressure in tissue fluids may be due to the inactivation of additional —SH and reducing groups supplied to the body, or due to an increase in physical solution and diffusion of oxygen so provided, in overcoming the anoxæmia caused by either decreased oxygen-carrying capacity of hæmoglobin (for example, with *para*-aminopropiophenone[3]), or by smooth muscle constric-

Table 1. Effect of Respired Oxygen Pressure on the Protective Actions of Cystamine and 5-Hydroxytryptamine against Acute Radiation* Lethality in Rats† and Effect of Added Pretreatment with Sodium Cyanide

Experiment	Respired gas (pressure in atmospheres absolute)	Survivors at 30 days (per cent)	Mean survival over 30 days (± *S.D.*)
1	Air (1 atmosphere)	6/9 (67)	25·90 (± 6·80)
	Oxygen (2 atmospheres)	6/10 (60)	24·90 (± 8·31)
	Oxygen (4 atmospheres)	7/9 (78)	25·00 (± 9·59)
	Oxygen (5 atmospheres)	4/10 (40)	21·60 (± 10·84)
2	Air (1 atmosphere)	6/9 (67)	26·10 (± 7·00)
	Oxygen (2 atmospheres)	6/10 (60)	22·10 (± 10·38)
	Oxygen (4 atmospheres)	1/9 (11)	13·00 (± 9·11)
	Oxygen (5 atmospheres)	2/9 (22)	12·11 (± 10·43)
3	Air (1 atmosphere)	5/10 (50)	22·90 (± 9·18)
	Oxygen (2 atmospheres)	4/10 (40)	20·30 (± 9·37)
	Oxygen (4 atmospheres)	2/10 (20)	13·90 (± 9·00)
	Oxygen (5 atmospheres)	0/10 (0)	8·60 (± 1·24)
4	Air (1 atmosphere)	2/10 (20)	17·90 (± 8·36)
	Oxygen (2 atmospheres)	2/10 (20)	14·10 (± 10·30)
	Oxygen (4 atmospheres)	1/10 (10)	11·40 (± 7·72)
	Oxygen (5 atmospheres)	0/10 (0)	9·50 (± 3·15)

Experiment 1. Rats pretreated with cystamine‡ (15 mgm.).
Experiment 2. Rats pretreated with sodium cyanide§ (2 mgm.) and cystamine‡ (15 mgm.).
Experiment 3. Rats pretreated with 5-hydroxytryptamine‡ (2 mgm.)
Experiment 4. Rats pretreated with sodium cyanide§ (2 mgm.) and 5-hydroxytryptamine‡ (2 mgm.).

* All animals received 1,000 r. X-rays whole-body irradiation.
† Rats of Canberra black stock weighing 150–190 gm., of equal sexes.
‡ Cystamine or 5-hydroxytryptamine administered as intraperitoneal injection 5 min. preceding irradiation.
§ Sodium cyanide administered intraperitoneally 5 min. prior to injection of protective agent (cystamine or 5-hydroxytryptamine).

tion in bronchioles or arterioles resulting from pharmacological action (for example, 5-hydroxytryptamine[4]). However, the protective disulphide, cystamine (—S—S—), is reduced to cysteamine (—SH), *in vivo*[5] and *in vitro*[6], and increased oxygen pressure *in vivo* may be expected to lessen its protective action, if the latter depends on the reduction of cystamine in tissues, to produce available —SH groups, or promote pharmacological activity, both of which tend to result in intracellular anoxia.

Our studies of the effect of respired oxygen tension on the protective action of cystamine and 5-hydroxytryptamine in rats subjected to acute lethal whole-body irradiation show that an increase in respired oxygen pressure to 4 and 5 atmospheres absolute pressure almost completely reverses the protective action of 5-hydroxytryptamine but fails to cause significant reduction in the protective action of cystamine. An example of the experimental results is given in Table 1. It is seen that pretreatment with sodium cyanide, itself a reducing substance but not protective in rats[7,8], results in a substantial reduction in the protective action of cystamine (and 5-hydroxytryptamine) if combined with increased oxygen pressure. In other experiments in which the dose of cystamine was reduced to 4, 8 and 12 mgm./rat respectively, raised oxygen pressures (4 atmospheres absolute) also failed to decrease the lesser protective action resulting and, indeed, tended to enhance the protective action to levels corresponding to a higher dosage of cystamine.

The results indicate that the protective action of cystamine (—S—S—) does not result entirely from the formation *in vivo* of cysteamine (—SH), although the enhancement of protective action at lower dose levels may support the hypothesis of Pihl and Eldjarn[9] that the degree of protective action is correlated with the ability to form mixed disulphides. That protective action for the disulphide depends on its histamine-liberating qualities[10] is not supported by our findings with histamine protection and its reversal by oxygen respired at 2 atmospheres absolute[8]. Again, the effect of combined cyanide pretreatment and oxygen pressure (Table 1) fails to support the theory of free-radical competition as applied to radioprotection *in vivo* of either cystamine or reducing substances, and a decrease of intracellular oxygen pressures (delayed by pretreatment with respiratory enzyme poisons) seems to be at least an important contributing factor. If the transfer of oxygen across cellular membranes involves active transport mechanisms, as distinct from simple physical diffusion, it may be easier to interpret chemical protection in terms of an 'oxygen effect'. These studies will be reported in full elsewhere.

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Effect of X-Rays on the Cell

It is well known that the inhibition of the synthesis of nucleic acid plays a very important part in the chain of the post-irradiation changes and particularly in the blocking of mitosis. But this hypothesis does not explain the great susceptibility of mitosis to comparatively small doses of irradiation[1]. The generalization relating sensitivity to mitotic activity fails when applied to lymphocytes. Other important biochemical processes in the cell, as, for example, the oxidative phosphorylation, are also sensitive to irradiation[2]. It cannot be excluded that other cellular functions are also impaired by X-rays.

We were able to demonstrate that, as yet, unknown changes[3] occur in tissue after small doses of irradiation. We incubated the tissue homogenate at 38° C. and found that coagulation occurred in a homogenate prepared from the organ of the irradiated rat, whereas coagulation did not occur in a similar homogenate prepared from tissue from a non-irradiated animal. We found this effect with the spleen, and also with 'radioresistant' liver tissue.

The coagulation phenomena may best be followed with the aid of turbidometric measurements. The present communication reports some properties of liver homogenates from irradiated and non-irradiated rats, studied by this method and by *p*H measurements.

The animals generally used in our experiments were Wistar albino rats of both sexes. We used a 'Super Sanax' X-ray apparatus operated at 180 kV. and 15 m.amp. with 1-mm. aluminium and 0·5-mm. copper filters, focus distance 53 cm., dose-rate 40 r./min. Animals were killed by decapitation 30 min.

after irradiation. The liver was removed after perfusion *in situ* with cold physiological saline and gently homogenized in distilled water (1 gm. in 10 ml.). We used a chilled loose Potter–Elvehjem glass homogenizer (about 20 up-and-down strokes of the pestle, driven at 1,500 rev./min.). Colorimeter tubes were filled with the whole homogenate, consisting chiefly of disrupted and partly of whole cells (after filtration through mill cloth) and the increase of optical density during incubation was measured against water using a green-yellow filter. In some experiments we measured the *p*H of the homogenate during the incubation (ionoscope apparatus) with antimony and calomel electrodes at 22° C.

We found that the coagulation of the homogenate from irradiated rats takes place more quickly in comparison with the coagulation of the control. It is interesting that it is possible to prove by this method the effect of a dose of 10 r. The speed of coagulation depends on the dose of irradiation (Table 1).

Table 1

Irradiation dose	Time of incubation at 38° C. required to reduce light transmission of homogenate by 50 per cent (average for 3 rats)
500 r.	265 min.
100 r.	295 min.
10 r.	375 min.
Control	425 min.

Raising the temperature of incubation accelerates the coagulation of the liver homogenate from the irradiated animal as well as that of the control. The *p*H in liver homogenate from irradiated animals falls more rapidly than that from controls (Fig. 1).

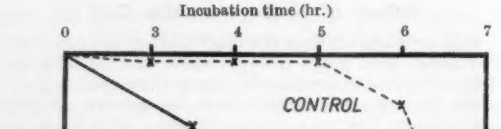

Fig 1. Drop in the *p*H of liver homogenate from irradiated and non-irradiated rats

We studied similar phenomena on spleen homogenate. It coagulates faster than liver homogenate, however, so that it is more difficult to demonstrate the influence of small doses of irradiation in that case.

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PATHOLOGY

Prevention of Adaptive Formation of Tryptophan Peroxidase by a Carcinogenic Azo Dye

The carcinogenic azodye 3′-methyl,4-dimethyl aminoazobenzene induces gross tumours in livers of rats fed a basal diet poor in riboflavin[1]. The whole process, in Sprague–Dawley rats, takes approximately 200 days. Structural damage to liver tissue may be detected histologically during the first weeks of carcinogen feeding[2]. Intracellular changes, manifested by the gradual depletion of mitochondria and ergastoplasm, which result in respiratory damage to the cell, can be detected by differential centrifugation weeks and even months before onset of malignancy[3]; yet all these changes are long-range effects. Even though the continual administration of this carcinogen brings about respiratory damage to the cell, the carcinogen is not a respiratory inhibitor[4,5]. It can be shown that the specific respiratory activity (activity per mgm. protein nitrogen) with sodium pyruvate as substrate remains normal for mitochondria during feeding with this carcinogen although the tissue is full of protein-bound azodye[6]. In short, the immediate chemical effect of this carcinogen, apart from its binding to one or more proteins not yet functionally characterized[6], is still obscure.

Assuming that the depletion of cytoplasmic structures may be due to the interference of the carcinogen with some phase of the mechanism of protein synthesis, we decided to study its effect on adaptive enzyme formation. It is known that tryptophan peroxidase is an adaptive enzyme formed in mammalian livers in increased amounts following the injection of L-tryptophan[7]. The enzyme is synthesized *de novo* by the liver cell from its constituent amino-acids[8]. It was reported recently[9] that there exists in the hepatoma a block in the pathway of tryptophan metabolism via kynurenin, indicating the absence of tryptophan peroxidase. There seem to be no results, however, in the literature on the level of this enzyme and its adaptive synthesis in the precancerous liver. For these reasons we investigated the induction of tryptophan peroxidase in livers of Sprague–Dawley rats (two months old at the beginning of the experiment) fed for various lengths of time on a basal diet containing 3′-methyl,4-dimethyl aminoazobenzene (twice recrystallized, 600 mgm./kgm. of food) as compared with controls on a basal diet without the carcinogen.

L-Tryptophan (78 mgm. in 10 ml.) was injected intraperitoneally to half the animals; the other half remained uninjected. 6 hr. after injection all animals were killed. The liver tissue was homogenized in isotonic neutral potassium chloride. Tryptophan peroxidase was assayed by the spectrophotometric method of Knox[10] from metaphosphoric acid extracts of samples incubated at 37° C. for 60 min. and unincubated controls. The sediments obtained after precipitation with 15 per cent metaphosphoric acid were extracted by Schneider's procedure[11] and the deoxyribonucleic acid was measured by the diphenylamine reaction. Assuming 5×10^{-12} gm. to be the constant deoxyribonucleic acid content of a rat diploid nucleus[12], the results expressed on this basis indicate the activity of tissue per nucleus.

Table 1 shows that there is no manifest change in tryptophan peroxidase activity in the course of 80 days of carcinogen feeding. On the other hand,

Table 1. EFFECT OF FEEDING 3'-METHYL,4-DIMETHYL AMINOAZOBENZENE ON ADAPTIVE SYNTHESIS OF TRYPTOPHAN PEROXIDASE

Time (days)			0	10	25	30	35	80
Basal diet	Non-injected	Activity	0·420	0·724	0·484	0·287	0·429	0·640
		DNA	702	740	570	544	700	576
		Spec. act.	0·300	0·489	0·368	0·263	0·300	0·556
	Tryptophan-injected	Activity	1·550	1·580	1·240	1·130	1·410	1·672
		DNA	786	552	500	584	700	524
		Spec. act.	0·987	1·436	1·240	0·966	1·007	1·024
		Increase	0·687	0·947	0·882	0·703	0·707	1·024
		Percentage	229	195	240	168	230	184
Basal diet plus carcinogen	Non-injected	Activity		0·620	0·384	0·366	0·420	0·440
		DNA		750	530	764	500	760
		Spec. act.		0·413	0·359	0·476	0·420	0·290
	Tryptophan injected	Activity		1·130	0·480	0·476	0·829	0·550
		DNA		675	530	572	700	692
		Spec. act.		0·836	0·452	0·417	0·440	0·410
		Increase		0·423	0·093	0	0·020	0·120
		Percentage		100	25			40

Activity = μmoles of kynurenin formed in 1 ml. homogenate in 1 hr. at 37° C. DNA = μgm. in 1 ml. homogenate. Spec. act. = activity per 5×10^{-12} gm. DNA.

the ability to form new tryptophan peroxidase after the injection of tryptophan declines rapidly in 10 days, and is later either zero or very low compared with the controls. Fractionation of tissue homogenized in isotonic sucrose has shown that tryptophan peroxidase is present only in the soluble phase of the cell, remaining in the supernatant after the components of the broken ergastoplasm have been spun down by centrifugation for 1 hr. at 59,000*g* in the 'Spinco' preparative ultracentrifuge. Thus, feeding the carcinogen, rather than inhibiting tryptophan peroxidase, prevents the cell from forming new soluble protein.

In order to decide whether the drop in adaptive enzyme formation is due to structural damage or to a block of the enzyme-forming system we administered single massive doses of the carcinogen (40 mgm. in 10 ml. corn oil) intraperitoneally to rats kept on a basal diet for four weeks prior to the experiment. The controls were injected with corn oil alone. 17 hr. after intraperitoneal injection half the control animals and half the experimental animals (3 of each) were injected with tryptophan. A parallel test indicated that in animals treated with the carcinogen a large amount of the carcinogen was protein-bound and could be liberated only after prolonged hydrolysis of tissue[6]. Assay, performed 6 hr. after injection of tryptophan, showed a substantial inhibition of tryptophan peroxidase formation in rats which received carcinogen.

We believe that these results provide evidence that 3'-methyl,4-dimethyl aminoazobenzene blocks the synthesis of tryptophan peroxidase, and possibly of other soluble proteins, by the enzyme-forming system of the hepatic cell.

Table 2. EFFECT OF A SINGLE INJECTION OF 3'-METHYL,4-DIMETHYL AMINOAZOBENZENE ON ADAPTIVE SYNTHESIS OF TRYPTOPHAN PEROXIDE

Corn oil	Non-injected (3 animals)	Activity	0·600
		DNA	744
		Spec. act.	0·400
	Tryptophan injected (3 animals)	Activity	1·300
		DNA	608
		Spec. act.	1 060
		Increase	0·660
		Percentage	165
Corn oil plus carcinogen	Non-injected (3 animals)	Activity	0·418
		DNA	530
		Spec. act.	0·400
	Tryptophan injected (3 animals)	Activity	0·612
		DNA	604
		Spec. act.	0·505
		Increase	0·105
		Percentage	25

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Enterotoxicity of Bacteria-free Culture-filtrate of *Vibrio cholerae*

Two Ogawa strains of *V. cholerae* maintained for six months in the laboratory by fortnightly subculture on agar slopes, and two Inaba strains maintained similarly for about eighteen months have been used for this study. All the strains, which were smooth, were checked for their identity and particularly for their inability to lyse sheep red blood cells. 5 ml. of an 18-hr. culture of each strain in Dunham's peptone water medium was aseptically transferred to a 1-litre Roux bottle containing 200 ml. of a sterile medium of the following composition and a *p*H of 7·6: 'Bacto-peptone' (Difco), 5·0 gm.; sodium chloride, 0·5 gm.; water, 100 ml.

The bottle was laid flat in the incubator, with a maximum free surface of the medium exposed to the air, for about 18 hr. The liquid culture was spun in a 'Servall' superspeed centrifuge in the cold room (2–4° C.) for 10 min. to throw down the bulk of the bacteria. The supernatant was cleared further

by high-speed centrifugation for 20 min. at about 14,000 r.p.m., at about 18° C. The clear supernatant fluid was filtered under suction through a Courtauld (later Oxoid) membrane filter. The filtrate was tested for sterility by inoculating 0·1 ml. into 2 ml. of each of Dunham's peptone water and of nutrient broth and incubating for about 42 hr. If unsterile, it was refiltered until sterile. 1 ml. of the sterile filtrate was injected slowly (taking 45–60 sec.) into the lumen of 4-in. loop of rabbit small intestine isolated by two silk ligatures. Essentially, a previous technique for studying the mechanism of action of *V. cholerae* on the intestinal mucous membrane was followed. A second loop of the same length in the same animal about one foot away from the first, was injected with the control medium (5 per cent peptone) treated in a similar way. Next morning, the animal was killed and changes in the loops were looked for. From the first preparation, three such experiments were performed. Two more preparations from the same (Ogawa) strain and three from each of the remaining three strains were tested once each on rabbit loops.

Each of the fourteen control loops was collapsed, and very little fluid, if any, could be expressed from inside them. All fourteen loops receiving culture filtrates were distended with fluid. Some appeared pale, in others blood vessels on the outer wall looked as if they had been injected, while still others appeared hæmorrhagic. The contained fluid was either like rice water, slightly pink, or definitely red. The volume of the contents was about 10 ml. and showed evidence of the presence of mucus, a few epithelial cells and a variable number of red blood corpuscles. The centrifuged supernatant was alkaline (*p*H 8–9) and had the following composition: nitrogen, 0·69 mgm./ml.; sodium, 144 m.equiv./l.; potassium, 5·6 m.equiv./l.; chloride, 85 m.equiv./l. Nitrogen was determined by Kjeldahl's method, the others by flame photometry. Cultures of scrapings from the mucous surface of the loops were negative to *V. cholerae*. The histological picture of the wall of the swollen loops was variable and included œdema of the mucosa and/or submucosa, infiltration of the mucous membrane with lymphocytes and occasional polymorphonuclear cells, congestion of the blood vessels and some denudation of surface epithelium. In places, the villi seemed to have disappeared altogether, leaving only a thin strip of mucous membrane still attached to the muscular wall. The control loops did not show any histological change.

Three filtrates prepared from an 18-hr. culture of an Ogawa strain in Dunham's peptone water medium, and one filtrate from each of the other three strains grown similarly did not produce any gross or microscopic change in the rabbit loops.

Further details of the activities of different filtrates will be published elsewhere. I am grateful to the Principal, Medical College, Calcutta, for permission to publish this work and to Dr. D. M. Bose, director, Bose Institute, for giving me full facilities of his laboratories. My thanks are due to Dr. Amitabha Sen for helpful criticisms, to Dr. M. L. Ghose for help in the animal experiments, and to Prof. M. L. Chakrabarty for carrying out the flame photometry.

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Influence of L-3′,5,3-Triiodothyronine on the Oxidative Phosphorylation of Tumour Mitochondria

The tumour cell differs from the normal cell mainly in its strong unco-ordinated growth. As any form of growth is combined with an increased consumption of energy, the tumour cell must consequently possess an increased energy metabolism compared with that of the normal cell. This increased expenditure of energy of the malignant cell cannot be completely explained by substrate oxidation via the tricarboxylic acid cycle, as the respiration of the tumour cell is no greater than that of the normal cell. According to Warburg[1] the respiration of tumour cells is even less than that of corresponding normal cells. As is well known, glycolysis, that is, the reduction of carbohydrates to lactic acid, which takes place also under aerobic conditions, is considered as a source of the additional energy production.

The synthesis of energy-rich phosphate bonds, which is coupled with respiration, is decisive for the energy production of the cell, that is, the so-called oxidative phosphorylation. According to Martius[2], Lehninger[3] and other authors, oxidative phosphorylation can be uncoupled by several substances. In this way the synthesis of energy-rich phosphates is disturbed and the cell suffers from energy deficiency.

Similarly, with regard to the energy metabolism of the tumour cell, it should be possible to disturb its energy balance with uncoupling substances and thus to retard the growth. Now, the vitality of a cell which is disturbed in its energy metabolism is impaired and that cell is inferior compared with other normal cells. Therefore, tumour cell damaged in this way should more easily succumb to the body's defence mechanisms and should also be more effectively influenced by cytostatica or radiotherapy. Starting from this hypothesis, we tested triiodothyronine, which was discovered by Martius[4] and Lehninger[5] to be an uncoupling agent of oxidative phosphorylation. As already reported[6], in optimal concentration it reduces tumour growth. Now it seemed to be essential to prove that triiodothyronine uncouples oxidative phosphorylation of tumour mitochondria, too. This, at first, does not appear to be obvious as its uncoupling effect depends on a 'swelling effect' of the mitochondria[5], but the membrane of tumour mitochondria differs considerably in its permeability from the membranes of normal mitochondria[7] and accordingly an uncoupling effect is not necessarily to be expected.

Table 1 shows the influence of triiodothyronine on the oxidative phosphorylation of mitochondria from Walker carcinoma. The mitochondria were isolated from necrosis-free tumour slices in an isotonic sucrose solution and were employed without previous treatment by triiodothryonine.

The comparatively high phosphorus/oxygen ratios of the tumour mitochondria where triiodothyronine was not added results from their greatly reduced respiration. This is possibly a consequence of the impaired respiration of tumour cells postulated by Warburg[1].

We consider that the considerable increase of X-ray sensitivity in experimental tumours and human cancers after administration of triiodothyronine, as recently demonstrated by Stein and Griem[8], can, partially at least, also be explained by a disturbance in the fundamental processes of oxidative phosphorylation. In addition, our experi-

Table 1. INFLUENCE OF TRIIODOTHYRONINE ON OXIDATIVE PHOSPHORYLATION.

The medium contained: 100 m.moles potassium chloride, 35 m.moles magnesium chloride, 10^{-3} m.moles cytochrome *c*, 12 m.moles ketoglutaric acid as substrate, 21 m.moles potassium dihydrogen phosphate, 10 m.moles adenosine monophosphate, 40 m.moles glucose, 30 units hexokinase and 50 m.moles histidine and hydrochloric acid to neutralise it. Triiodothyronine $7{\cdot}5 \times 10^{-5}$ moles, *p*H 7·4. Each deposit contained also 0·5 mgm. or 2·0 mgm. diphosphopyridine nucleotide. The respiration of the mitochondria was measured according to the Warburg technique, temperature 28° C., duration of experiment 20 min. The phosphate was determined according to Fiske and Subbarow (ref. 8)

Triiodothyronine added	DPN added (mgm.)	O_2 uptake (μmoles)	P (μmoles)	P/O	Inhibition (per cent)
No	0·5	2·0	4·6	2·3	—
Yes	0·5	2·1	0·64	0·3	87
No	2·0	2·5	3·5	1·4	—
Yes	2·0	3·7	0·31	0·1	93

ments on various malignant neoplasms lead us to believe that the sensitivity of tumours to cytostatica is also considerably increased experimentally and clinically by combined treatment with uncoupling agents like triiodothyronine. A more detailed report of this work will be published later.

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A Cytopathogenic Agent from a Mammary Tumour in a *C3H* Mouse that produces Tumours in Swiss Mice and Hamsters

Stewart, Eddy *et al.*[1-3] recently reported that an agent derived from leukæmic *AK* mouse tissue produces a cytopathogenic effect in cultures of mouse embryo cells. Furthermore, after passage in tissue culture, this agent, which they term the S.E. Polyoma virus, produces a variety of tumours when it is injected into newborn Swiss mice and hamsters. This report describes observations on an agent with similar properties but derived from a different source.

Tissue from a mammary tumour in a *C3H*/Jax retired breeder was homogenized in a Potter–Elvehjem homogenizer and centrifuged at low speed. The supernatant was frozen. Later it was thawed, and 0·3 ml. added to a single Carrel flask culture of normal *C3Hf*/HeHa mouse kidney cells maintained in a medium consisting of *CMRL*-1066[4] supplemented with 20 per cent horse serum. An uninoculated sister culture was kept as control. The media were changed on the third and ninth days. A cytopathogenic effect was first observed in the inoculated culture on the twelfth day, and was extreme on the thirteenth day. The culture fluid was centrifuged gently, and 0·1 ml. of the supernatant was added to each of several cultures of mouse kidney cells. Cytopathogenic effects were then observed after 4 days. Ten further consecutive passages were made and at each passage a cytopathogenic effect was seen 3–6 days after the culture was inoculated.

The virus is cytopathogenic for normal kidney cells from two different sublines of *C3H* mice (*C3H*/StHa and *C3Hf*/HeHa), and for kidney cells and embryo cells of Swiss *ICR* mice. It has no apparent effect on kidney cells of monkey or rabbit. It remains cytopathogenic after being kept at − 10° C. for 3 months, or at 37° C. for 3 hr., or at 57° C. for 30 min. It has caused hæmagglutination of normal rabbit and guinea pig erythrocytes in titres to 1 : 2560.

Tissue culture fluid from the fifth passage was injected into 50 Swiss mice on the first or second day after birth. The mice exhibited early stunting and poor hair growth similar to that described in newborn mice injected with the polyoma virus[2]. 45 mice survived to weaning. Tumours were observed from the fourth month after injection. To date, nine months after injection, 16 mice have developed a total of 18 tumours. Eleven had parotid tumours, 3, all virgin females, had mammary adenocarcinomas, 2 had thymic lymphomas, and one had all three types.

53 newborn Swiss mice were injected with fluid from normal cultures of *C3Hf* kidney cells. Of these, 48 survived to weaning. No stunting was observed in these mice. Five months after injection, a parotid tumour was observed in one, and to date 3 have developed parotid tumours, and one had both a parotid tumour and a thymic lymphoma. The fact that these mice developed tumours suggests that they received some virus. There are at least three possible ways that this could have occurred: (1) the control cultures could have become contaminated with virus from the inoculated cultures; (2) tumour virus could have been present in the *C3H* tissues used to initiate the control cultures, and this would be consistent with the observation of Gross[5] that extracts of normal organs of *C3Hf* mice injected into newborn mice of the same strain can induce parotid tumours; or (3) the newborn mice might have become infected by air-borne virus. In considering these possibilities it should be mentioned that Mirand[6] has also observed tumours to develop in newborn mice injected with control culture material, and Buffett *et al.*[7] have shown that tumours sometimes develop in animals kept in close contact with others that were injected with subcellular particulates from tumours.

Ten of the tumours in our experimental group have been examined in the electron microscope. In two of these, large numbers of extracellular virus-like particles were observed (Fig. 1), and in four others, smaller numbers were seen. The particles were about 110 mμ in diameter, and many had a dense, central nucleoid body. No particles were seen in the remaining tumours. Three tumours that appeared in mice injected with fluid from normal cultures have been examined, and no particles have been found.

Virus from the twelfth passage was injected into newborn Syrian hamsters. A high percentage have already developed tumours, with deaths occurring from these tumours as early as ten days after injection.

Thus we have obtained, from a *C3H* mouse mammary tumour, a cytopathogenic virus with broad tumour-inducing properties. This may be a new tumour virus. But at least two other possibilities must be considered. First, our findings are so

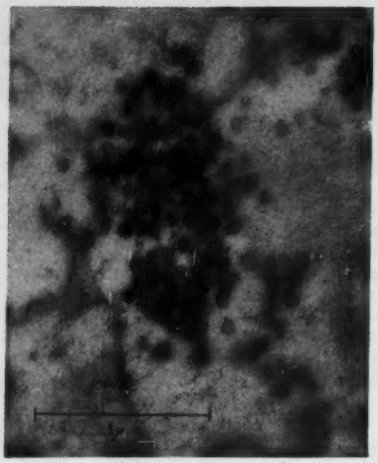

Fig. 1. Electron micrograph showing virus-like bodies in a parotid tumour induced in a Swiss mouse by the cytopathogenic tumour virus derived from *C3H* mouse mammary tumour

similar to those of Stewart and Eddy that they raise the question of whether or not we are dealing with the same virus as theirs. Since we were not carrying the polyoma virus in our laboratory at the time when the injections were made, our preparations could not have been contaminated with polyoma virus. The polyoma virus was obtained from leukæmic tissue of *AK* mice and ours was obtained from a *C3H* mouse mammary tumour. Therefore, if our virus is indeed the polyoma virus, it would mean that the *C3H* mammary tumour and the *AK* leukæmic tissue must have contained the same virus. There is no direct experimental evidence bearing on this point. Secondly, since the tumour from which our extract was made contained the milk agent[8,9] it is possible that this latter virus induced the variety of tumours which we observed. If so, we must assume that passage in tissue culture increased the pathogenicity of the virus to account for the wide tumour-inducing properties not hitherto associated with the milk agent. Finally, it is possible that our results could be accounted for by not one, but a mixture of viruses.

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MICROBIOLOGY

Production of Acid from Arabinose and Xylose by Strains of *Azotobacter*

During investigations on the utilization of pentoses by *Azotobacter*, production of acid was observed in many cases in media with arabinose or xylose as sole source of carbon. This acid production is easily demonstrated by inoculation of nitrogen-free agar plates containing 0·5 per cent xylose and 0·5 per cent glucose and undissolved calcium carbonate. In this medium a distinct clear zone develops around the colonies (Fig. 1).

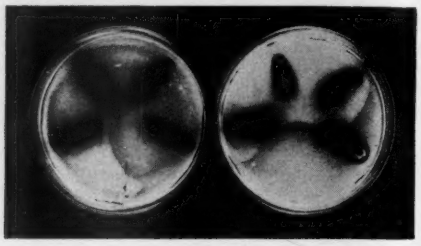

Fig. 1. Nitrogen-free agar plates with 0·5 per cent xylose and 0·5 per cent glucose and undissolved calcium carbonate, inoculated with 10 *Azotobacter* strains, of which eight show acid production

The *Azotobacter* strains were partly stock cultures kept from earlier investigations and partly new isolates from samples of garden soil. The basal medium used had the following composition (gm.): KH_2PO_4, 0·2; K_2HPO_4, 0·8; $MgSO_4 \cdot 7H_2O$, 0·2; NaCl, 0·2; $CaSO_4 \cdot 2H_2O$, 0·1; $Fe_2(SO_4)_3$, 0·01; $Na_2MoO_4 \cdot 2H_2O$, 0·005; 'Bacto' agar 1·0, in 1,000 ml. distilled water, *p*H 7·2. The pentoses were added in concentrations of 0·5 per cent.

*p*H determinations were made with a glass electrode after 14 days incubation at 25° C. The results are recorded in Table 1. Most of the cultures showed no visible growth. Only 5 strains of *A. chroococcum* and 1 strain of *A. beijerinckii* were able to grow well with arabinose as sole carbon source, and xylose was not utilized at all by these species. The strains of *A. vinelandii*, on the other hand, were better able to utilize both arabinose and xylose, although visible growth was in no case accompanied by acid production.

In subsequent experiments with 3 strains of *A. vinelandii*, production of acid, breakdown of xylose, and nitrogen fixation were measured simultaneously (Table 2). The acid produced was determined by back titration to the initial *p*H. Xylose was determined by the method of Somogyi, and nitrogen by the Kjeldahl method. The strains *P*-3 and *P*-4 showed

Table 1. *p*H Values of Cultures on Pentose Media after 14 Days Incubation at 25° C.

	Number of strains lowering *p*H to				
	4·0–4·4	4·5–4·9	5·0–5·4	5·5–5·9	6·0–7·1
Arabinose					
A. chrooc.	0	4	0	2	20
A. beij.	0	0	0	0	7
A. vinel.	0	0	0	0	10
Total	0	4	0	2	37
Xylose					
A. chrooc.	18	6	0	0	2
A. beij.	4	1	0	0	2
A. vinel.	5	0	0	1	4
Total	27	7	0	1	8

Table 2. Decomposition of Xylose, Fixation of Nitrogen and Production of Acid by Strains of *A. vinelandii* in Media with Xylose as Sole Source of Carbon. (Initial Xylose Content about 100 mgm.)

Strain	Final *p*H	Acid produced (m.equiv.)	Nitrogen fixed (mgm.)	Xylose decomposed (mgm.)	Equivalent acid/moles xylose	Mgm. nitrogen fixed/gm. xylose decomposed
P-3						
Without	4·40	0·117	0	17	1·0	0
$CaCO_3$	4·37	0·117	0	17	1·0	0
With	—	—	0	92	—	0
$CaCO_3$	—	—	0	92	—	0
P-4						
Without	4·34	0·119	0	21	0·9	0
$CaCO_3$	4·38	0·121	0	23	0·8	0
With	—	—	0	87	—	0
$CaCO_3$	—	—	0	85	—	0
P-5						
Without	6·89	—	0·45	55	—	8·2
$CaCO_3$	6·90	—	0·45	56	—	8·0
With	—	—	0·47	56	—	8·4
$CaCO_3$	—	—	0·47	54	—	8·7

no visible growth and no nitrogen fixation could be detected, but distinct production of acid had taken place with simultaneous disappearance of xylose. In cultures without calcium carbonate this process was soon inhibited by the acid reaction, and only about one-fifth of the xylose was decomposed with production of about 1 equivalent of acid/mole of xylose. In cultures with calcium carbonate, however, four-fifths or more of the xylose was decomposed. Unlike these two strains, *P*-5 was able to utilize xylose and showed rather good growth. No production of acid had taken place and growth was equally good with or without calcium carbonate.

The decomposition of carbohydrates by *Azotobacter* is not normally accompanied by production of acid, but acid formation may take place in certain circumstances, and a few cases have been recorded previously[1]. Jensen proposes as a possible explanation that strains of *Azotobacter* can oxidize certain carbohydrates to the corresponding sugar acids, but are unable to metabolize these acids further, and this theory is supported by the ratio between acid production and carbohydrate dissimilation in the above experiments. The enzymes concerned with this initial oxidation are probably less specific than those concerned with the later steps of decomposition. No proof of these theories is available yet, but at least it seems clear that the breakdown of carbohydrates by *Azotobacter* follows a scheme different from that of Embden–Meyerhof, with production of acid in one of the initial reactions.

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The Phenylalanine Test on Kligler's Iron Agar for the Identification of *Proteus*

Buttiaux and his colleagues[1] have questioned the reliability of the urea test for the identification of members of the genus *Proteus* in routine investigations of Enterobacteriaceae. They found many urease-negative *Proteus* strains, and, using Stuart's urea medium[2] they, moreover, detected many urease-positive strains of the genus *Paracolobactrum*.

The Providencia group, now renamed *Proteus inconstans* in the latest edition of "Bergey's Manual of Determinative Bacteriology"[3], is generally urease-negative. All *Proteus* strains, however, are able to deaminate phenylalanine oxidatively to phenylpyruvic acid, a reaction which other members of the Enterobacteriaceae are unable to effect. Buttiaux and his fellow-workers consider the phenylalanine test, which demonstrates this deamination, to be a much more reliable characteristic of *Proteus* than the urea test. Henriksen's[4] method of performing the phenylalanine test, and that of Buttiaux and his colleagues, described in the paper cited above, are too complex and time-consuming for many laboratories. Ben Hamida and Le Minor[5] devised a simple and rapid test using harvested cells from an agar slope, shaken in a buffered solution of phenylalanine; phenylpyruvic acid being tested for, after 10 min. shaking on a Kahn shaker, by the addition of a ferric chloride solution. An intense green coloration was positive, for the presence of phenylpyruvic acid.

This technique seemed admirably suited to adaptation, preferably with the view of extending the use of another diagnostic medium such as Kligler's iron agar, the cells washed from such a culture serving as the suspension for the phenylalanine test.

Forty or so strains of various members of the Enterobacteriaceae, including 18 in the genus *Proteus*, were inoculated on to slopes of Kligler's iron agar, the medium being stabbed to the foot of the tube from the lower angle of the slope.

This method was preferred to the usual one of stabbing the butt from the centre of the slope, for two reasons: (1) the yellow reaction from a positive glucose dissimilation did not spread to the upper half of the tube; consequently no false positive lactose reactions occurred due to incomplete acid reversal on the slope; (2) the blackening of the medium due to the formation of hydrogen sulphide was similarly confined to the butt.

After stabbing, the slope was well streaked out to ensure maximal surface growth for the performance of the phenylalanine test.

The reactions in the Kligler's tubes were read after 18–24 hr. incubation at 37° C. and the cells from each slope were then washed off with 0·5 ml. of a 0·2 per cent L-phenylalanine solution in 0·1 molar phosphate buffer to *p*H 7·4, the cells being removed from the slope into the buffer solution by rubbing with a wire-loop, to obtain the heaviest suspension. This suspension was then poured into a bijou bottle and shaken for 10 min. on a 'Microid' flask shaker.

The shaking was then stopped, 0·1 ml. of an 8 per cent aqueous ferric chloride solution added to the suspension, and the bijou reshaken for 30 sec. or so.

An intense dark green colour was produced in the presence of phenylpyruvic acid. In every case only the *Proteus* strains gave this positive reaction. The other strains produced yellow or brownish reactions. The results obtained with the strains using the phenylalanine method of Buttiaux *et al.*[1] agreed entirely with these results.

The presence of sulphides in Kligler's medium did not interfere with either the performance or the reading of the test.

The advantage of using one medium for as many reliable tests as possible is obvious when one considers the increased risks of contamination involved in taking two 'picks' from any one colony on a selective plate, to inoculate two tubes of media.

To inoculate a separate slope for the phenylalanine test from a cultured Kligler slope means that there is an 18–24 hr. delay before a rapid phenylalanine test, such as Ben Hamida and Le Minor[5] suggested, can be carried out.

The extremely simple extension in the use of Kligler's medium outlined above to include the phenylalanine test should prove of value where speed of diagnosis is essential.

Other multiple sugar media could presumably be so utilized instead of that of Kligler.

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A Group of Pseudomonads able to synthesize Poly-β-hydroxybutyric Acid

Poly-β-hydroxybutyric acid has been found to occur very infrequently in *Pseudomonas* although it is very common in the related genera *Chromobacterium*, *Spirillum* and *Vibrio*[1,2]. It is absent in authentic strains of *P. aeruginosa*, *P. fluorescens*, *P. aureofaciens*, *P. convexa*, *P. schuylkilliensis*, *P. pavonacea*, *P. lemonnieri*, *P. mildenbergii*, *P. syncyanea* and *P. chlororaphis*. Of classified strains tested it only appears to be present in the animal and the plant pathogens, *P. pseudomallei*[3] and *P. solanacearum*, which are scarcely typical of the genus. It has also been found in *P. antimycetica*[4] and a group of related organisms, which appear to be pseudomonads, referred to in previous publications[1,2] as "pseudomonads related to Hankey Culture Collection (HCC) B175". This group has now been studied in some detail and should, we feel, be considered members of *Pseudomonas*.

The strains were isolated mainly from Trinidad soils on glucose–'Marmite'–peptone agar plates. They form characteristic opaque yellow colonies which in some strains change after 2–3 days to an intense reddish-purple colour. They are all non-sporing, non-acid-fast, Gram-negative rods, 0·7–1·3μ × 0·4μ, occurring singly or in pairs; motile by means of a polar tuft of flagella varying from 3 to 8 in number. They are aerobic. They grow well at 37° C. and to some extent at 42° C. They will not grow below 15° C. All strains rapidly liquefy gelatin and peptonize milk. Citrates are utilized as the sole source of carbon. Neither indole nor acetoin is formed. Cellulose is not attacked.

The group is characterized by the production of massive inclusions of poly-β-hydroxybutyric acid on media containing a readily available energy source, and the production of a diffusible fluorescent pigment in asparagine–salts media[5] giving a violet fluorescence in ultra-violet light easily distinguishable from the bluish-green fluorescence of the normal fluorescent pseudomonads listed above. The strains all grow rapidly in a lactate salts medium. They have an oxidative metabolism[6] and rapidly oxidize glucose, sucrose and lactose. Their fermentative ability is weak—slight acid, no gas, from glucose but not from other sugars. They are strongly lipolytic. They hydrolyse æsculin but not starch.

They form two clearly defined sub-groups on the basis of pigment formation and nitrate reduction (Table 1).

Production of the purple pigment was examined. The best medium for pigment production was glycerol–peptone (2 per cent glycerol, 1 per cent peptone ('Oxoid'), 0·5 per cent sodium chloride, 0·1 per cent potassium nitrate). Strong aeration is essential. 1 litre of the above medium was inoculated with strain *B*175 and grown for three days in shake flasks. The purple pigment is insoluble in water and the usual organic solvents. The cells and pigment were therefore recovered by centrifuging and dried. The dried cells (about 10 gm.) were extracted with chloroform in a solvent extraction apparatus for 18 hr. The chloroform solution contained a small quantity of a yellow-green pigment and 3·1 gm. of poly-β-hydroxybutyric acid, m.p. 168° C. The residue was blended with 95 per cent ethanol containing 2 per cent v/v concentrated hydrochloric acid. This treatment causes the insoluble purple pigment to change to a yellow form soluble in organic solvents.

The purple form can be re-formed by making the solution alkaline. The alcohol extracts were concentrated to small volume and water added. The yellow pigment was extracted from the water layer with chloroform. The chloroform solution was chromatographed on silica gel, considerable dark-coloured material remaining adsorbed while pure pigment was eluted. The pigment was recrystallized from a benzene–*cyclo*hexane mixture as orange-yellow

Table 1

Strain No.	Strain Name	Source	Colour on glucose–'Marmite'–peptone–agar plate (5 days)	Nitrate reduction (5 days)	Growth at 37° C.	Growth at 42° C.
1	*B*175	forest soil	dark red/purple	–	+++	+
2	Maraval	river water	red/purple	–	+++	+
3	*N.C.R.*	forest soil	brown/purple	–	+++	+
4	43	river water	purple	–	+++	++
5	*S*11*	soil	brown/purple	–	+++	++
6	Santa Cruz	cocoa soil	red/purple	–	+++	+
7	*N.C.R.*2	forest soil	yellow/purple	–	+++	+
8	*N.C.R.*3	forest soil	violet	–	+++	+
9	*N.C.R.*4	forest soil	violet	–	+++	+
10	*T*590	soil	red/purple	–	+++	+
11	*T*400	soil	red/purple	–	+++	+
12	Rupununi	savannah soil	yellow	+	+++	++
13	*T*277	heavy clay soil	yellow	+	+++	+
14	Centeno 6	savannah soil	yellow	+	+++	+++
15	Longdenville	citrus soil	yellow	+	+++	+
16	*T*456	soil	yellow	+	+++	++
17	Hololo	cocoa soil	yellow	+	+++	+
18	C.E.S.	savannah soil	yellow	+	+++	+

* This isolate has already been studied by Sneath (designated by him 71, ref. 7)

needles, m.p. 237–238° C. (2·5 per cent yield). (Found: C, 58·0; H, 3·8; O, 28·7; N, 8·5; OCH_3, 9·3; NCH_3, 8·9 per cent; molecular weight, 340. Calculated for $C_{16}H_{12}N_2O_6$: C, 58·5; H, 3·7; O, 29·2; N, 8·5; 1-OMe, 9·4; 1-NMe, 8·9 per cent; molecular weight, 329.) Hydrolysis of the pigment with 2 *N* sodium hydroxide for 20 hr. yielded a red acid, m.p. 284° C. Zinc dust distillation of this acid gave a yellow-brown distillate from which phenazine (identified by melting point and ultra-violet spectrum) was isolated by crystallization and sublimation. The original pigment depressed the melting point of an equal quantity of phenazine-α-carboxylic acid by 40 deg. C., indicating the non-identity of the two compounds. The absorption spectrum of the pigment differs markedly from the spectra of violacein, pyocyanin, chlororaphin, iodinin, phenazine-α-carboxylic acid[8], and the blue pigment from *P. lemonnieri*[9]. The chemical structure of this new phenazine pigment is being studied.

The organisms are therefore a group of Gram-negative, polar-flagellated rods of oxidative metabolism. They produce a diffusible fluorescent substance and the pigmented members produce phenazine pigments. Following Tobie[10], they should be included in the genus *Pseudomonas*.

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GENETICS and CYTOLOGY

Two New Basic Chromosome Numbers in the Musaceae

The scheme proposed by Cheesman[1], and now generally accepted, for the classification of the Musaceae depends partly on basic chromosome numbers. Two genera are recognized. The genus *Ensete* Horan. comprises species with monocarpic habit and $2n = 18$ chromosomes. Species of *Musa* L. form suckers more or less freely and are placed in four sections: Musa (Eumusa) and Rhodochlamys with $2n = 22$, Callimusa and Australimusa with $2n = 20$. This scheme includes nicely all the species thus far described, but among recent introductions to the Trinidad collections are two exceptional forms for which the classification will need to be elaborated.

Somatic chromosome counts were made from root tips by the method of Tjio and Levan[2].

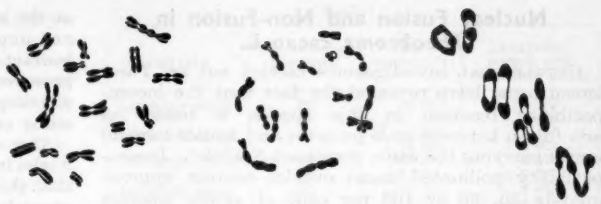

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Somatic chromosomes of *I.R.* 503. (× 2,300)
Fig. 2. Somatic chromosomes of *I.R.* 510. (× 2,300)
Fig. 3. First meiotic metaphase in *I.R.* 503, showing a chain of four and seven bivalents. (× 2,300)

The first introduction, *I.R.* 503, is a seed collection from British North Borneo and is fairly certainly the unknown species noted by Simmonds[3], on the basis of herbarium material, as probably a new Rhodochlamys. Living plants, however, are more reminiscent of Callimusa; they differ from Callimusa spp. in seed characters and in the chromosome number, which is $2n = 18$ (Fig. 1). The second, *I.R.* 510, comes from the highlands of north-central New Guinea and is probably the largest herb in existence[4]. It has largish seeds, reminiscent of *Ensete*, but in some other respects resembles *Musa*. In its sparse suckering, especially if the leading stem is damaged, it is more or less intermediate between the two genera. The chromosome number is $2n = 14$ (Fig. 2). In neither case has the proper taxonomic position been decided, but at least one new section and perhaps a new genus can be envisaged. Indeed, it appears that a study of the wild bananas of the Indonesian area, still partly unknown, might enlarge the family considerably.

Only *I.R.* 503 has so far flowered in Trinidad and meiosis has been examined in acetocarmine smears of pollen mother cells of one plant. Chains of three or four chromosomes were seen at metaphase in occasional cells, indicating heterozygosity for a small translocation (Fig. 3). Structural diversity of this kind is frequently encountered in *Musa*. Translocation hybridity has been reported in cultivated clones of Eumusa, in hybrids between 22-chromosome species and in hybrids between geographical races of *M. acuminata*[5–7]. More recently, I have found (unpublished work) that homozygous translocations are distributed among the geographical sub-species and races of *M. acuminata* on a scale comparable with that found in *Gaillardia*[8], and have noted multivalents in several new crosses of 22-chromosome species and in one Australimusa hybrid. However, despite this high frequency of occurrence in edible bananas and in experimental hybrids, the present case is the first in which heterozygosity has been recorded in a wild collection. There is no evidence as to whether the translocation is a recent one or has been revealed by the mingling of already diverse populations.

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Nuclear Fusion and Non-Fusion in *Theobroma cacao* L.

Cytological investigations carried out on *Theobroma cacao* have revealed the fact that the incompatibility reaction in this species is based on non-fusion between male gametes and female haploid nuclei carrying the same dominant S allele[1]. Incompatibility pollinated cacao ovaries contain approximately 25, 50 or 100 per cent of ovules wherein fusions between the first male gamete and the egg nucleus and between the second male gamete and the polar nuclei have not taken place. In such ovules, the egg and male nuclei appear always to lie in actual contact, each preserving its spherical shape; the second male gamete and the polar nuclei, however, do not show such regularity of behaviour, for the two polar nuclei may sometimes become dissociated from one another, the male nucleus lying elsewhere in the embryo-sac or in contact with one of the separated polar nuclei.

When fusion does take place between a male gamete and the polar nuclei, the male gamete invariably places itself at some point on the line of juncture of the two polar nuclei, so that the male nucleus lies in simultaneous contact with both female nuclei before fusion takes place. The male gamete appears not to fuse at random over the surface of one or other of the polar nuclei but always comes into the position where all three nuclei are in mutual contact. The male nucleus then insinuates itself between the polar nuclei, assuming a more and more fusiform shape as it penetrates. Finally, the contents of the male nucleus mingle with the nucleoplasm of one of the polar nuclei. The process is shown in diagrammatic form in Fig. 1.

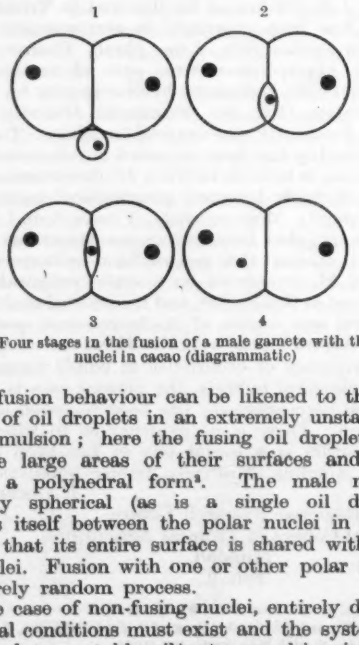

Fig. 1. Four stages in the fusion of a male gamete with the polar nuclei in cacao (diagrammatic)

This fusion behaviour can be likened to the coalescence of oil droplets in an extremely unstable oil/water emulsion; here the fusing oil droplets tend to share large areas of their surfaces and hence assume a polyhedral form[2]. The male nucleus, originally spherical (as is a single oil droplet), arranges itself between the polar nuclei in such a manner that its entire surface is shared with these two nuclei. Fusion with one or other polar nucleus is a purely random process.

In the case of non-fusing nuclei, entirely different interfacial conditions must exist and the system can be likened to a stable oil/water emulsion in which no coalescence of the oil droplets takes place. In emulsions stabilized, for example, with a protein, direct oil-to-oil contact between droplets is prevented by the presence of a monolayer of adsorbed protein at the surface of each droplet. This condition does not imply that the stabilized droplets cannot make contact, but rather that when contact is made they preserve their spherical form. Clusters of spherical oil droplets can be seen under the microscope in many emulsions.

The not infrequent separation of the two polar nuclei in ovules showing non-fusion leads us to suggest that this effect is brought about by the presence of a strongly but specifically adsorbed substance in the cytoplasm of the male nucleus carrying the dominant S allele. When both male and polar nuclei carry the same dominant allele (for example, S_1 nuclei in in $S_{1\cdot3}$ selfed or in $S_{1\cdot2} \times S_{1\cdot3}$), the substance which 'interferes' with nuclear fusion diffuses from the male cytoplasm into that associated with the polar nuclei, from which it interacts with specific sites on the surface of both polar nuclei, leading to protection against fusion (or stabilization of all three bodies). In these circumstances the separation of the nuclei is understandable.

No specific adsorption sites, apparently, are present at the surface of polar nuclei which carry the recessive allele (for example, S_3 nuclei in the $S_{1\cdot3}$ genotype) for fusion with any male nucleus will always take place. When the polar nuclei carry a dominant S allele different from that of the male gamete, fusion again will ensue; for example, S_3 polar nuclei in an $S_{3\cdot4}$ genotype will fuse with S_1 male gametes derived from an $S_{1\cdot2}$ genotype because the S_3 sites are not specific adsorption sites for the S_1 protective substance. If the male nucleus does not carry a dominant S allele, no protective substance is present in its cytoplasm; it will therefore always fuse with any female nuclei.

In view of the specificity of the non-fusion reaction, the protective substance is likely to be proteinaceous in character.

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Genetics of Dieldrin Resistance in *Lucilia cuprina* Wied.

In a comprehensive paper on insecticidal resistance in anopheline mosquitoes Davidson[1] demonstrated that resistance to dieldrin in *Anopheles gambiae* is due to a single gene, partially dominant. In this work the discriminating dose technique, supported by Milani[2], for genetical studies on insecticidal resistance in insects was utilized.

Employing this method in a study of the genetics of dieldrin resistance in *Lucilia cuprina*, in which insecticidal resistance has been demonstrated[3], it is indicated that dieldrin resistance in this insect is also due to a partially dominant single gene. To date information is available only from a cross between susceptible female flies and a single resistant male fly. The results, however, conform so closely to a Mendelian interpretation on a single gene basis that it can be expected that additional work will merely confirm the above conclusion.

In this preliminary study a single resistant male fly, which survived 4 μgm. of dieldrin, was crossed with ten susceptible female flies. The male fly was taken from a strain of flies undergoing selection in an

attempt to develop a homogeneous strain of blow-flies for genetical studies.

The male and female *F*1 progeny of the initial cross were intermediate in resistance. A dosage of 0·1 μgm. dieldrin/fly (the dose which consistently kills all susceptible flies) gave no mortality, while 4 μgm. dieldrin/fly gave complete mortality. Application of the discriminating dose (0·1 μgm. dieldrin/fly) on both males and females of the *F*2 resulted in close agreement with a 1 : 3 ratio (1 dead : 3 alive). The tests were made on 1,007 flies. The above ratio applied more closely to female than male flies.

In the back-crosses an approximate 1 : 1 ratio and complete survival resulted when the discriminating dose of 0·1 μgm. of dieldrin was applied to the progeny of *F*1 females × susceptible male (287 flies) and *F*1 females × resistant male (136 flies) respectively. Flies of both sexes were again treated.

The insecticide, dissolved in kerosene, was applied topically with a micro-applicator which delivered 0·5 μl./fly.

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Amounts of Deoxyribonucleic Acid in the Silk Glands of the Spider *Nephila maculata*, Fabr., 1763

The major chemical components of a chromosome so far described are two types of protein and deoxyribonucleic acid. The amounts of the two types of chromosomal protein vary considerably between the tissues of one animal[1]. Deoxyribonucleic acid content is much less variable, however, and appears[2–4] to be approximately constant in the nuclei of the same species. However, recent work by Leuchtenberger and Schrader[5] indicates that the quantities are likely to vary in secretory tissues.

In the course of this investigation the silk glands of *Nephila maculata* were selected for analysis. There are five types of silk glands in this spider, namely: (1) aggregate glands; (2) ampullate glands; (3) cylindrical glands; (4) aciniform glands; and (5) pyriform glands. All these glands are highly secretory and are concerned with the production of silk for the construction of the web.

The tissues were removed from freshly killed animals as quickly as possible and were fixed in Carnoy's acetic alcohol (3 parts of absolute alcohol : 1 part of glacial acetic acid). The fixation period was usually 4 hr. The fixative was then thoroughly washed off the tissues, which were then embedded in paraffin, cut at varying thickness, and stained with Feulgen stain as described by Stowell[6].

Measurements were made on an apparatus similar to that described by Pollister and Moses[7], with an *RCA* 931*A* electron multiplier phototube and a light source consisting of a tungsten ribbon filament lamp, from which the 546-mμ wave-length was isolated by the Farrand interference filter (peak 550 mμ) to estimate the Feulgen reaction. Readings were taken on a 'Rubicon' galvanometer. The final values in arbitrary units were obtained as described by Swift[8].

Table 1

Cell type	No. of nuclei measured	Volume of nuclei (μ^3)	Deoxyribonucleic acid (arbitrary units)
Aggregate gland	49	36·810 ± 11·453	2·339 ± 0·444
Ampullate gland	49	44·525 ± 15·058	2·495 ± 0·477
Cylindrical gland	49	29·981 ± 6·082	2·475 ± 0·397
Aciniform gland	49	31·535 ± 6·260	2·435 ± 0·461
Pyriform gland	50	42·662 ± 13·28	2·891 ± 0 595

Table 1 gives the results obtained by photometric estimations.

It will be seen that there is considerable variation in the volume of the nuclei from different silk glands. Even in the nuclei of the same gland the deviation is quite significant. This is indicative of the differential secretory activity in the nuclei, as the volume of the nuclei will be directly related to the accumulation of the products of secretion liberated into the cytoplasm. On the other hand, the quantities of deoxyribonucleic acid are remarkably constant not only in the nuclei of the same gland but also in the nuclei of the different glands. This indicates that the quantities of deoxyribonucleic acid are not directly related to the secretory activity of the nucleus. This finding is in agreement with the hypothesis of constancy, but differs from the observations of Leuchtenberger and Schrader[5], who find that the quantities of deoxyribonucleic acid in *Helix*, though generally constant in body tissues, are likely to vary in secretory tissues. The decrease of deoxyribonucleic acid in the nuclei of the salivary gland of *Helix* is, according to Leuchtenberger and Schrader, the result of its utilization in the formation of secretory products in the cytoplasm. In *Nephila*, however, the amount of deoxyribonucleic acid is constant and does not bear any direct relation to the secretory activity.

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HYDROLOGY

Marine Studies in the Inshore Waters of Antarctica near Mawson, 1956

This investigation, aimed at obtaining information concerning the hydrology and biology of the inshore waters of Antarctica, especially during the winter period, formed part of the scientific programme of the 1956–57 Australian National Antarctic Research Expedition to Mawson (67° 31′ S. lat., 62° 53′ E. long.) in MacRobertson Land. After a period of establishment, I began regular observations in June and continued at weekly intervals until February

1957. Two stations were occupied, one in 25 m. and the other in rather more than 100 m. of water. Observations were made at various depths throughout the water column. For the greater part of the period the surface was frozen to a maximum recorded depth in September of 115 cm. Water samples were taken in the normal manner with Nansen bottles but using modified operating techniques to meet the severe conditions. Plankton hauls, mainly vertical and entailing considerable difficulty, were run with *N*50 and *N*70 nets.

From the water samples, and in addition to records of temperature, measurements were obtained of *p*H, E_h, buffer capacity, dissolved phosphates and oxygen, and chlorinity. Measurements of submarine solar radiation were taken down to a maximum depth of 10 m. A high-speed continuous centrifuge was used to extract phytoplankton both for the determination of chlorophyll and for qualitative and quantitative examination.

Although the results are too extensive to be described in detail in this communication, it may be of interest to indicate briefly some of the features of particular interest. Chlorinity values obtained during winter were low, mean figures ranging from 16·00 to 18·70‰. These chlorinities, combined with mean temperatures between −1·90° C. and −1·60° C., commonly produced freezing conditions extending well down into the water column and appeared to cause the formation of ice crystals in free suspension, apart from the sea ice formed at the surface. Chlorinities were appreciably higher during summer except for a temporary shallow surface zone of extremely low salt content produced by the melting of the sea ice. Evidence was obtained which indicates a process by which the summer waters undergo incomplete mixing and remain close to the coastline until their replacement by relatively homogeneous water of higher chlorinity with the onset of the following summer.

As might be expected in these latitudes, the incidence of submarine solar radiation was found to exhibit marked seasonal differences, being negligible during the long winter period partly because of the low elevation of the Sun and partly because of the highly reflecting layer of sea ice. Consequent upon such conditions, phytoplankton was virtually absent during the winter period although small numbers of zooplankton organisms were taken in the nets. It is of interest that phytoplankton appeared and began to increase and attached diatoms were found actively growing on the under surface of the sea ice while the ice itself was more than 100 cm. thick. Large-scale blooms did not occur, however, until the ice was in an advanced stage of disintegration during the thaw.

The striking qualitative differences between the phytoplankton extracted with the continuous centrifuge and that taken with the nets was particularly illuminating, especially as the greater part of the chlorophyll estimated must have been contributed by forms either rare or absent in the material gathered by conventional methods. Based on chlorophyll concentrations, it was estimated that the carbon content in the standing crops of summer phytoplankton lay between 0·30 and 14·80 mgm./m.³.

Full details of the above investigation will be published elsewhere.

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ARCHÆOLOGY

Apple-Scoops

Being a private collector of items relating to bygone dentistry, I was naturally interested to read Prof. Raymond A. Dart's communication (*Nature*, March 21, p. 844) on "An 'Australopithecine' Scoop from Herefordshire".

Apple-scoops (occasionally termed gouges) were freely used in apple-growing districts by edentulous persons to scrape small thin pieces off the fruit and thus facilitate mastication. This custom was particularly prevalent prior to the introduction into Great Britain, in 1855, of vulcanite as a base for artificial dentures. Until then, such restorations were expensive due to the time and labour necessary to carve them from hippopotamus or walrus tusks with the precision that would ensure an accurate fit: the alternative being natural or ivory teeth attached to a swaged gold foundation.

The most popular apple-scoops were undoubtedly home-made and fashioned from the metacarpus or metatarsus of sheep. Others were constructed of ivory. A silver (Birmingham hallmark 1830) specimen, which I possess, unscrews from its hollowed ivory handle and fits into this for portability. Incidentally, there is a small but very comprehensive collection of apple-scoops in the Folk Museum, Gloucester.

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METALLURGY

Ductile Cleavage Fracture of Molybdenum

Most metals other than those having a face-centred cubic structure show a more or less rapid transition from ductile to brittle behaviour as the temperature is lowered. Well above the transition temperature the fracture surfaces are mainly fibrous with only a few cleavage facets, but at intermediate temperatures fracture occurs by cleavage after a strain of a few per cent. So far little attention has been paid to the mechanism of this ductile cleavage fracture. Recently, we have examined cleavage facets and longitudinal sections of a number of fractured specimens in which cleavage occurred after straining about 10 per cent at room temperature at a rate of strain of $0{\cdot}88 \times 10^{-4}$ sec.$^{-1}$. The tensile specimens, which had grain diameters of about 2 mm., were prepared from arc-cast deoxidized molybdenum and were 0·1 in. in diameter[1]. The results throw some light on the mechanism of fracture.

Part of a typical fracture edge is shown in Fig. 1. It is made up mainly of straight-line segments which often occupy one complete grain (see *A* in Fig. 1). Within about 0·5 mm. of each fracture edge a number of subsidiary cracks are observed (see *B* in Fig. 1) and often these are arranged in a characteristic configuration, an example of which is shown in Fig. 2. The cracks do not cross grain boundaries and those making up a configuration are always contained in one grain. Occasionally an 'ear', such as that shown at *C* in Fig. 1, is observed on the edge of the fracture. Such ears have been shown to be the result of the joining of two overlapping cracks on different cleavage planes by the shearing of the material

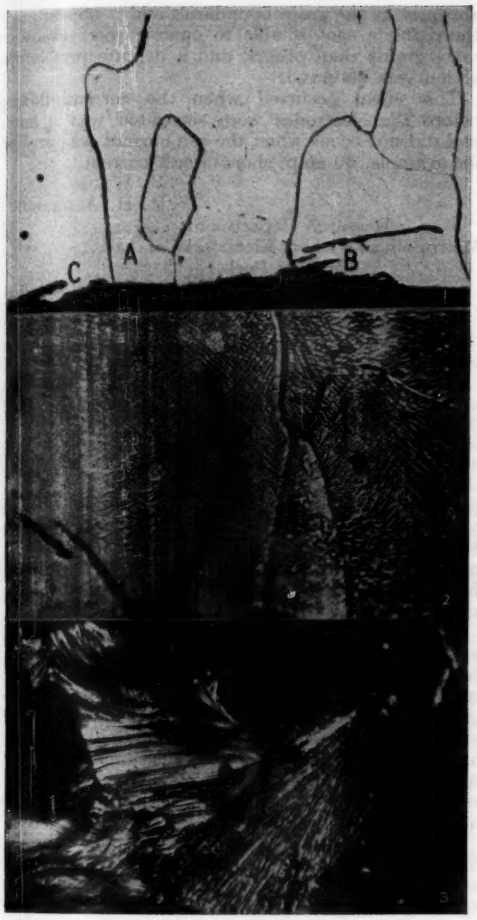

Fig. 1. Longitudinal section through the fracture surface of a molybdenum specimen which fractured after 10 per cent strain at room temperature. (× 250)

Fig. 2. A similar specimen to that of Fig. 1, but electro-etched, showing wavy slip lines and 'forced slip' near the grain boundaries. (× 250)

Fig. 3. Photograph of some cleavage facets on the fracture surface of a specimen similar to those shown in Figs. 1 and 2. (× 250)

between them[2]. The markings on the cleavage facets (Fig. 3) suggest that the growth directions are different for each crack.

We believe that these results show that fracture occurs by the joining of many cracks which grow co-operatively in a narrow band across the specimen. The grain boundaries provide barriers which are sufficient to prevent their propagation, and the final stage of the fracture is one of shearing at or near the grain boundaries to form the complete fracture surface. Some of the cracks are not needed to form the fracture surface, and these are left after fracture as subsidiary cracks such as those in Figs. 1 and 2. The existence of configurations of cracks such as that in Fig. 2 shows that cracks can occur on more than one crystallographic plane, even within one grain.

The wavy slip lines shown in Fig. 2 were revealed by electro-etching and are characteristic of body-centred cubic metals. Near the grain boundaries, 'forced slip' has occurred on intersecting slip systems and this would be expected to increase still further the difficulty of propagating a crack through a grain boundary.

A more detailed study of this type of fracture is being made and will be reported elsewhere. The work is being carried out while one of us (A. A. J.) is holding a University of London I.C.I. Research Fellowship and the other (B. J. S.) a U.K. Atomic Energy Authority Research Bursary.

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A New Silicide in a 12 per cent Chromium Steel

In the course of an investigation into the tempering characteristics of a 12 per cent chromium steel (C 0·098, Mn 3·18, Si 3·02, Ni 4·06, Cr 12·56, Mo 0·49, V 1·09 per cent) a new phase was discovered. This phase (which we have called *H*-phase) could not be obtained in isolation but was always associated with $M_{23}C_6$. A residue extracted after tempering the steel for 50 hr. at 750° C., however, gave the maximum proportion of the new phase to $M_{23}C_6$. This was of the order of 2 : 1. This residue was examined in more detail by X-rays and a full chemical analysis was obtained.

The specimen was photographed in a 9-cm. diameter powder camera and a quadruple focusing camera, cobalt $K\alpha$ radiation being used in both cases. The interplanar spacings of the new phase fitted a cubic lattice with $a = 10{\cdot}735$ kX. The interplanar spacings are listed in Table 1, together with line intensities and indices. The X-ray pattern of the new phase shows marked resemblances to the pattern of M_6C. The space group of the new phase is probably *Fd3m*.

The full chemical analysis, converted to atomic percentages, is shown in Table 2. Due to the presence of $M_{23}C_6$ it is very difficult to form any definite conclusions about the composition of the new phase

Table 1. X-Ray Data for *H*-Phase ($a = 10{\cdot}735$ kX.)

Intensity	$h^2 + k^2 + l^2$	Corrected *d*	Observed *d*
vw*	11	3·237	—
vw*	12	3·099	—
vw*	16	2·689	—
vw	19	2·463	2·439
wm	24	2·191	2·176
s	27	2·066	2·051
wm	32	1·898	1·887
wm†	36	1·789	1·782
vw	44	1·618	1·612
w	51	1·503	1·497
w	59	1·398	1·394
vw	67	1·311	1·307
ms	72	$1{\cdot}265_1$	$1{\cdot}261_6$
wm	75	$1{\cdot}239_0$	$1{\cdot}235_9$
vw	84	$1{\cdot}171_3$	$1{\cdot}169_1$
m	99	$1{\cdot}078_9$	$1{\cdot}078_5$
wm	104	$1{\cdot}052_7$	$1{\cdot}051_6$
w	107	$1{\cdot}037_6$	$1{\cdot}037_1$
w	108	$1{\cdot}033_0$	$1{\cdot}031_7$
w	123	0·9679	0·9680
vw	132	0·9344	0·9342
wm	136	0·9205	0·9204

* Detected on the focusing camera film only.
† Interference due to an $M_{23}C_6$ line.

Table 2. Analysis of a Mixture of *H*-Phase and $M_{23}C_6$ (in the Approximate Ratio of 2 : 1)

Element	Atomic weight
C	8·8
N	2·8
Mn	0·8
Ni	4·0
Cr	18·8
Mo	1·2
V	8·2
Fe	20·8
Si	34·6

but the large amount of silicon is significant. After allowing for the $M_{23}C_6$ the new phase appears to be a silicide of formula MSi_x, where M stands for Fe, Cr, V, Ni and Mo, and x is probably greater than 1.

The full elucidation of the composition and structure of this phase must await further attempts to isolate or synthesize it.

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PHYSICAL SCIENCES

An Effect of Oxide Layers on the Behaviour of Vacuum Arc Cathode Spots

In the course of a preliminary experiment to study the effect of electrode surface condition on the behaviour of the cathode spot of a d.c. vacuum arc an interesting phenomenon was observed.

The surface of a vacuum-cast copper cathode was prepared by abrasion on emery paper and hand polishing on a 'Selvyt' cloth. After degreasing in a petroleum ether still, the surface was cathodically etched in argon and the grain boundaries were clearly revealed. The surface was then oxidized to cuprous oxide by heating in air.

The d.c. arc discharge was struck by touching a pointed tungsten anode on to the prepared surface, both electrodes being mounted in a glass vacuum chamber exhausted to 0·01μ mercury pressure. A mechanical switch shorted the arc after 20 millisec.

The cathode spots left characteristic continuous tracks on the electrode surface, and it was found that the markings were absent from certain grains as shown in Fig. 1. The characteristic tracks stop abruptly at the grain boundaries and it appears that the cathode spot is able to operate more easily on some grains than others, and a definite preferential action was observed.

This effect occurred when the current flowing before the electrodes were separated was 5 amp., and did not occur when the arc current was greater, for example, 50 amp. short-circuit current.

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The λ-Transition in Helium

Recent experiments on the inelastic scattering of neutrons by superfluid helium[1,2] have given a direct confirmation of the correctness of the elementary excitation spectrum (rotons and phonons) of Landau's theory[3]. As it is usually formulated, the theory postulates that the excitation spectrum is independent of temperature. However, the velocity of sound is known to vary somewhat with temperature[4], and the neutron scattering experiments[1] show an appreciable fall in the minimum roton energy Δ, as the temperature approaches the λ-point.

It is possible to understand the non-existence of superfluidity above the λ-point if one has reason to believe that Δ vanishes at the λ-point[5]. But if Δ really were to vanish at the λ-point, it would almost certainly imply the existence of an Ehrenfest[6] second-order transition, that is, a finite discontinuity in the specific heat. Recent measurements of the specific heat of liquid helium[7] show a more complicated anomaly—a finite discontinuity is superimposed on a logarithmic infinity.

A phenomenological model can easily be constructed for the dependence on temperature of Δ, leading to a specific heat anomaly of the kind observed, while agreeing closely with the observed $\Delta(T)$ within the temperature region covered by the neutron scattering experiments[1]. One such model for $\Delta(T)$ is :

$$\left.\begin{aligned} \Delta &= \Delta_0, && \xi < -1 \\ &= \Delta_0(|\xi| + \xi\ln|\xi| + e^{-2})/(1+e^{-2}), && -1 < \xi < e^{-2} \\ &= 0, && \xi > e^{-2} \end{aligned}\right\} \quad (1)$$

where

$$\xi = \alpha(T/T_\lambda - 1), \quad (2)$$

and the constant of proportionality α is to be chosen to give the best fit with experiment.

Fig. 1 illustrates the postulated behaviour of Δ (where the scale factor α in equation (2) has been put equal to 4); for comparison the experimental points of Larsson and Otnes[1] are also shown. The specific heat anomaly arises from the singularity in $d\Delta/d\xi$ at $\xi = 0$. This derivative has a finite discontinuity and a logarithmic infinity, and the ratio of the coefficient of the logarithm to the magnitude of the discontinuity is $\frac{1}{2}$. The experimental value[7] of this ratio is $\sim 0{\cdot}3$.

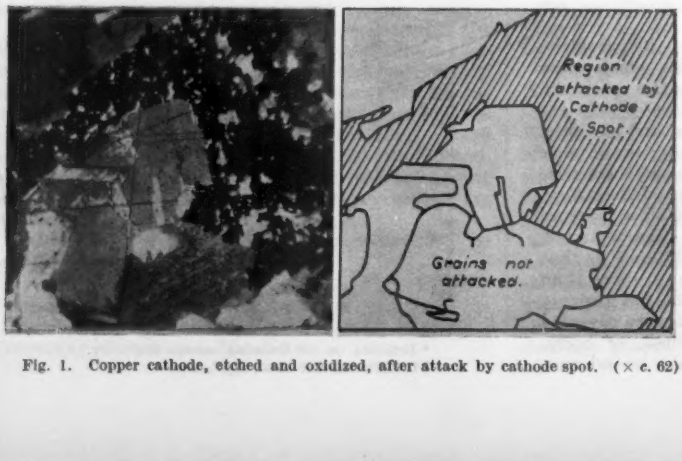

Fig. 1. Copper cathode, etched and oxidized, after attack by cathode spot. (× *c.* 62)

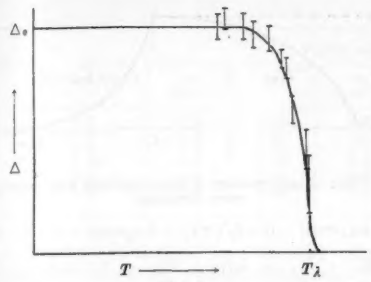

Fig. 1

If this phenomenological model has any significance, it implies that Δ does not vanish at the transition, but falls rapidly to zero for values of ξ slightly greater than zero (up to $\xi \sim 0{\cdot}1$). It might be hoped that inelastic neutron scattering experiments in a temperature region containing the λ-point (say, 2·0 to 2·3° K.) could test the present suggestion; unfortunately the results reported suggest that the line width becomes prohibitively great near the λ-point.

The form of $\Delta(\xi)$ may well be more complicated than is proposed here—equation (1) has discontinuous second derivatives at $\xi = -1$ and at $\xi = e^{-2}$ which would give spurious Ehrenfest third order transitions at these temperatures. If Δ is indeed finite above the λ-point, then the value of Δ can fulfil the role of the short-range order parameter in an order–disorder transition. The absence of superfluid behaviour immediately above the λ-point will, however, require a separate explanation, and may possibly be linked with the increasing line-width for neutron scattering.

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Townsend's First Ionization Coefficient in Pure Nitrogen

With equipment assembled for the measurement of Townsend's α in highly pure hydrocarbon gases, it was thought interesting to carry out preliminary but careful measurements in a common diatomic gas. Now that satisfactory agreement has been reported for hydrogen[1,8] which does not depend too critically on purity, it was hoped to confirm the latest results in pure nitrogen obtained by Harrison[2], which differ from all other workers (and those to be presented here) by up to an order of magnitude.

Previous results[3–6], corrected to 0° C., are shown in Fig. 1. Excepting the values of Ayers[3], overall agreement is reasonable (within 30 per cent, later results agreeing better) considering the unspecified nature of possible contaminants present in each case. As a reference, the values of Masch will be used[4] because, among other reasons to be given, his results provide a consistent set of values taken over a wide pressure range and cover the whole E/p_0 region under consideration. Bowls's[6] values, though obtained with a mercury-free system, only cover the higher E/p_0 region.

As shown, Harrison obtained values for (*a*) 99·99 per cent nitrogen, obtained from sodium azide; (*b*) 99·9 per cent purified cylinder nitrogen (0·06 per cent oxygen, 0·04 per cent argon, obtained by passing gas (*c*) through hot copper and a liquid nitrogen trap); (*c*) 99·9 per cent cylinder nitrogen. His results indicate that: (1) the presence of 0·1 per cent of common impurities such as oxygen or argon lowers α/p_0 by from 30 to 3 per cent; (2) the presence of a further $\simeq 0{\cdot}1$ per cent of some other contaminant found in cylinder nitrogen and removed by liquid nitrogen lowers α/p_0 by another 35 per cent. These rather large effects would satisfactorily account for previously found discrepancies and would indicate that Masch's results should compare favourably with Harrison's (*a*) curve. Comparing the absolute magnitude of these two sets of results, however, reveals a discrepancy by a factor of 2–10; even using Harrison's (*c*) results still gives a discrepancy of 2–6 times. A shift in the E/p_0 axis of about 10 would give almost perfect agreement all round.

Values of mine obtained in 99·998 per cent nitrogen are also shown in Fig. 1 (impurities: $O_2 < 10$ vol./million; $CO_2 < 5$ v./m.; $H_2 < 1$ v./m.; $A \leqslant 5$ v./m.; gas supplied by B.O.C. Ltd.). These were obtained in a ¾-litre system incorporating ultra-high vacuum techniques and which will be described elsewhere. To make optimum use of the variable 0·2–1·2 cm. parallel-plate gap, the pressure of the samples covered 50–400 mm. mercury. Mercury contamination was completely eliminated using a differential pressure device. Conditions were such that no impurity greater than that originally present

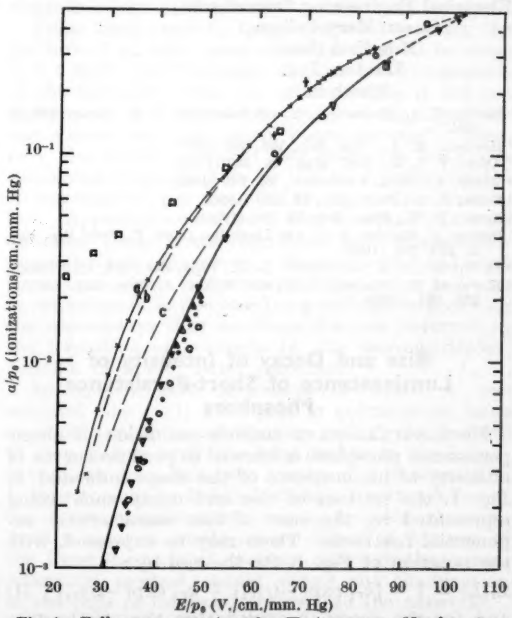

Fig. 1. Full curve, present work; □, Ayers; ▽, Masch; ○, Posin; +, Bowls; △, Dutton, Haydon and Llewelyn Jones; ●, de Bitetto and Fisher; ×, Harrison (*a*, *b*, *c*)

in the sample could possibly have been added. The use of a liquid-nitrogen trap in the gas supply line did not affect the results.

It is seen that the curve coincides with the values of Masch except at very low E/p_0. This agreement may seem surprising as the results of Masch were obtained in 99 per cent nitrogen in a mercury-contaminated system, which it is known increases the α/p_0 values. Although the presence of an impurity, as shown above, becomes more marked as E/p_0 decreases, the experimental means of obtaining these lower E/p_0 values necessitates for any particular system a corresponding use of increased gas pressures. In the case of Masch this pressure increase was exceptional, as he used a 6-mm. maximum gap. Thus, at $E/p_0 \simeq 100$, $p_0 \simeq 10$; at $E/p_0 \simeq 50$, $p_0 \simeq 700$ and at $E/p_0 \simeq 30$ a pressure of 2,000 mm. mercury was employed. At these pressures the effect of the presence of mercury should be small to negligible. A sharp break in the results of Posin[5] at $E/p_0 = 54$ at which the pressure employed was increased from 1 to 100 mm. mercury can also be attributed to the reduced effect of mercury contamination in the lower E/p_0 region. To check his gas samples Masch tested nitrogen obtained from sodium azide (as for Harrison's (*a*) curve) and could detect no difference.

Results obtained by N. J. Peacock (private communication) for 99·9 per cent cylinder nitrogen in a system with which excellent agreement was obtained for 99·95 per cent hydrogen[8], also agree with Masch's values and coincide with the present results. This agreement is important, as it enables α values obtained in necessarily large systems as a preliminary to the investigation of secondary processes to be used with confidence.

Thanks are due to Prof. M. W. Humphrey Davies for facilities provided. This work was carried out during the tenure of an Imperial Chemical Industries Fellowship, which is gratefully acknowledged.

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Rise and Decay of Intensity of Luminescence of Short-Persistence Phosphors

Rectangular-wave cathodo-excitation of short-persistence phosphors is known[1] to produce curves of intensity of luminescence of the shape indicated in Fig. 1, the sections of rise and decay each being represented by the sum of two characteristic exponential functions. These may be expressed, with the notation of Fig. 1, for the rise by:

$$L_r/L_e = 1 - [k_{r1}\exp(-t/\tau_{r1}) + k_{r2}\exp(-t/\tau_{r2})] \quad (1)$$

and for the decay, provided that the steady-state condition L_e has virtually been attained at $t < t_p$, by:

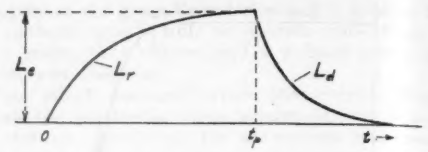

Fig. 1. Pulse of luminescence of short duration with rectangular-wave excitation

$$L_d/L_e = k_{d1}\exp[-(t-t_p)/\tau_{d1}] + k_{d2}\exp[-(t-t_p)/\tau_{d2}] \quad (2)$$

where $k_{r1} + k_{r2} = k_{d1} + k_{d2} = 1$, and $\tau_{r2} \ll \tau_{r1}$, $\tau_{d2} \ll \tau_{d1}$.

The short-period rise and decay of intensity of luminescence in phosphors of the respective types are commonly explained in terms of the random time an electron of a luminescence centre spends in an excited state. So far, no explanation appears to be available for the co-existence of the two exponential processes, with $\tau_2 \ll \tau_1$, during rise and decay.

The phenomenon that $\tau_2 \ll \tau_1$ may possibly be interpreted as the effect of a difference in the structures of luminescence centres, depending on whether they are situated in the body of a phosphor crystal or at, or near, its surface, and resulting in a considerable difference in the respective average time which an electron spends in an excited state. Measurements with phosphors of different crystal-sizes are to be made in order to clarify this question.

The rise and decay characteristics of the intensity of luminescence of the following short-persistence cathode-ray tube phosphors have been determined:

Phosphor *A*: ZnS: 0·005 per cent Ag
Phosphor *B*: ZnS: 0·005 per cent Ag: 0·0005 per cent Ni
Phosphor *C*: ZnS: 36 per cent CdS: 0·017 per cent Ag

(The phosphors were supplied by Derby Luminescents, Ltd.) Their spectral emission characteristics have the usual bell-shaped form with the peak at $\lambda = 0{\cdot}46\mu$ for phosphors *A* and *B*, and at $\lambda = 0{\cdot}525\mu$ for *C*. The phosphors were laid as screens in aluminized cathode-ray tubes of the flash-tube type[2], which were operated at an accelerator voltage of 15 kV. with rectangular-wave beam-current pulses of 30 μsec. duration at a frequency of 50 pulses/sec., the whole screen area of a tube being excited simultaneously and at a uniform density. The variations of intensity of luminescence were picked up with an electron-multiplier photovalve responding to radiation with $0{\cdot}30\mu < \lambda < 0{\cdot}65\mu$, and recorded with a high-speed recurrent-transient oscillograph.

The measurements were made for different values of electron beam density, J_b, and with the spectral response of the photovalve (*a*) corrected to that of the photometric standard observer for photopic vision, that is, eye correction, and (*b*) modified with a Chance *OX*7 filter so that the photovalve responded only to any ultra-violet radiation with $0{\cdot}30\mu < \lambda < 0{\cdot}43\mu$ transmitted through the cathode-ray tube face. The results are given in Table 1, the coefficients k and time constants τ relating to equations 1 and 2. The values of k_{r1} and k_{r2}, and of τ_{r2} (see equation 1) are not given in Table 1, because they could not be determined accurately due to the fact that the beginnings of the rising curves were not accurately recorded with the existing equipment.

The results in Table 1 confirm[1] that $\tau_{d2} \ll \tau_{d1}$, and that increasing J_b tends to reduce the values of τ_{d1} and τ_{d2}. In addition, it is to be noted that (*a*) in all cases we have $\tau_{d1} < \tau_{r1}$, and (*b*) the values of τ

Table 1

Phosphor	J_b (m.amp./cm.²)	Visible radiation					Ultra-violet radiation				
		τ_{r_1} (μsec.)	kd_1	τd_1 (μsec.)	kd_2	τd_2 (μsec.)	τ_{r_1} (μsec.)	kd_1	τd_1 (μsec.)	kd_2	τd_2 (μsec.)
A	8·0	10·0	0·80	4·8	0·20	0·4	7·0	0·80	4·2	0·20	0·2
	2·0	11·2	0·80	7·0	0·20	0·5	10·0	0·80	5·2	0·20	0·2
B	5·0	8·5	0·88	5·5	0·12	1·0	4·5	0·80	4·0	0·20	0·4
	1·25	11·5	0·88	7·0	0·12	1·1	10·0	0·80	4·2	0·20	0·4
C	8·0	6·0	0·80	5·5	0·20	0·3	2·5	0·80	2·0	0·20	0·2
	3·0	10·0	0·80	7·0	0·20	0·5	7·0	0·80	5·5	0·20	0·9

for ultra-violet radiation are smaller than the corresponding values for visible radiation.

The difference in the values of τ for ultra-violet and visible radiations indicates a difference in the average time an electron spends in an excited state. It may perhaps be concluded that the difference depends on the height of the energy-level of the excited state and hence the magnitude of the energy-jump of the electron when returning to the ground-state, a higher energy-level being associated with a shorter average time in the state of excitation, and vice versa. This point is to be investigated in more detail.

The observation that $\tau_d < \tau_r$ contradicts existing theoretical considerations[1], according to which the time constant for rise should be smaller than that for decay.

Take approximations for the luminescence intensity curve (Fig. 1). Then for a rise in luminescence:

$$L_r/L_e = 1 - \exp(-t/\tau_r) \quad (3)$$

and for decay, with L_e independent of t_p,

$$L_d/L_e = [1 - \exp(-t_p/\tau_r)] \exp[-(t-t_p)/\tau_d] \quad (4)$$

The energy, W_r, stored in excited electrons during the rise is, from equation 3 and with a proportionality factor A,

$$W_r = A\int_0^{t_p}(L_e - L_r)\,dt = A\,L_e\,\tau_r[1 - \exp(-t_p/\tau_r)] \quad (5)$$

The energy, W_d, converted from excited electrons to luminescent radiation during decay is, from equation 4:

$$W_d = A\int_{t_p}^{\infty} L_d\,dt = A\,L_e\,\tau_d\,[1 - \exp(-t_p/\tau_r)] \quad (6)$$

From equations 5 and 6:

$$\tau_d/\tau_r = W_d/W_r \quad (7)$$

Because of non-radiative dissipation of energy of excited electrons when returning to the ground-state, we have $W_d < W_r$, and thus from equation 7 that $\tau_d < \tau_r$, which is in accordance with experimental evidence.

The magnitude of the ratio τ_d/τ_r is governed by the magnitude of non-radiative dissipation of energy and is therefore a measure for the probability of non-radiative return of an excited electron to the ground-state.

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Manchester College of Science and Technology.

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[2] Dudley, M. D., Whitney, I., and Feinberg, R., *Nature*, **179**, 1024 (1957).

Estimation of Chain Fracture during the Cross-linking of Rubber Hydrocarbons by High-Energy Radiation

Exposure of hydrocarbon polymer molecules to high-energy radiation results either in a decrease in their molecular weight or intermolecular interaction, with the eventual formation of an insoluble network. The primary factor which determines what happens is the relative incidence of fracture of the main chain of carbon atoms to cross-link formation. The ratio of fractures to cross-links, β, has usually been estimated from studies of the solubility of irradiated polymers. The bulk of such work has been on polyethylenes, and even under comparable experimental conditions widely differing estimates of β have been reported. Measurements of the stress relaxation during irradiation have also been used in an attempt to obtain alternative estimates of the chain fracture during irradiation[1].

One of us has recently developed an analysis which permits the calculation of both degree of cross-linking and the molecular weight of primary molecular chains cross-linked into the network from simple extension stress–strain measurements[2]. This analysis requires the determination of two elastic constants C_1 and λ^*; the value of C_1 depends upon both the frequency of cross-links and the molecular weight of primary molecular chains, while the value of λ^* depends solely on the former.

These three methods have been used to study the fracture of purified natural rubber exposed *in vacuo* to a 4-MeV. electron beam. No quantitative analysis of the solubility data was attempted as it did not conform to the behaviour predicted by Charlesby and Pinner from theoretical considerations[3]. However, the results indicated that there was little consummated chain fracture as the sol fraction tended to less than 0·7 per cent after long periods of irradiation. Approximate values of β of 0·14 and 0·11 were obtained from stress–relaxation and stress–strain measurements respectively. The value of β obtained from stress–strain measurements may prove to be an under-estimate, as it is based on a calibration involving the assumption that no chain fracture occurred in the formation of networks by the thermal decomposition of di-*tert.* butyl peroxide.

An approximate calculation in which it was assumed that: (1) all bonds in polyisoprene have an equal probability of fracture and (2) the fracture of each carbon–hydrogen bond results in the formation of one cross-link gave a value of β of about 0·5. It thus appears probable that chain fracture followed by direct recombination was a frequent reaction. Such a reaction would escape detection by any of the three methods of analysis, although the stress–relaxation method should detect the combination of the ends of fractured chains into the network[4].

These three methods of analysis are now being used to investigate both the effect of additives and

the temperature of irradiation on the value of β, and experimental data will be published in the near future.

Samples were irradiated at Wantage Radiation Laboratories (Atomic Energy Research Establishment), and we thank Dr. R. Roberts and members of the Technological Irradiation Group for their kind co-operation.

L. Mullins
D. T. Turner

British Rubber Producers' Research Association,
48 Tewin Road,
Welwyn Garden City.
April 27.

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Etch Pits on Calcite Cleavage Faces

In a recent communication, Watts[1] describes a number of etch pits found in freshly cleaved calcite, and in particular notes that three, four- and five-sided pits are found, with the first two types having some curved sides. I have also observed similar shapes, and have examined these under a surface-finish interference microscope (C. Baker of Holborn, Ltd.) using a × 40 objective and a mercury vapour lamp with a green filter (λ = 0·564μ). This type of interferometer compares the surface under investigation with a reference surface in the objective, and shows a magnified image of the surface crossed by fringes. These fringes are straight for a perfectly plane surface, whereas irregularities cause the fringes to be shifted into a pattern that may be likened to a contour map, of vertical spacing 0·273μ. The number and direction of the fringes in the field may be varied by tilting the specimen.

All the investigations were on calcite plates that had been freshly polished to a high optical standard parallel to a cleavage plane, or parallel to the optical axis. These plates were first examined by various interferometric and optical transmission methods, and only those plates selected for the investigation in which no internal optical irregularities of any kind could be detected.

The surfaces were etched with 10 per cent hydrochloric acid for 10–60 sec., and then washed in distilled water.

Fig. 1*a* shows a cluster of four-sided etch pits in a surface parallel to a cleavage plane. The typical concave nature of the walls is clearly shown by the form of the fringes. A similar etch pit is shown in Fig. 1*b*, but with a single isolated pit in one wall of approximately an eighth of a micron deep. Fig. 1*c* shows a line of overlapping five-sided pits. Several lines of fringe disturbances appear to originate from the vicinity of the pits. It is possible that the line of pits may be due to preferential etching along a dislocation line.

The remaining photographs are of etch pits obtained on surfaces of plates cut parallel to the optical axis of the crystal. In this case, the pits are boat-shaped, as shown in Fig. 1*d*. With longer etching, the ends become more rounded (Fig. 1*e*), but the pits never appear to become circular. If the microscope stage and the specimen are tilted, the fringes can be made to cross a pit, as in Fig. 1*f*, to show its typical rounded bottom.

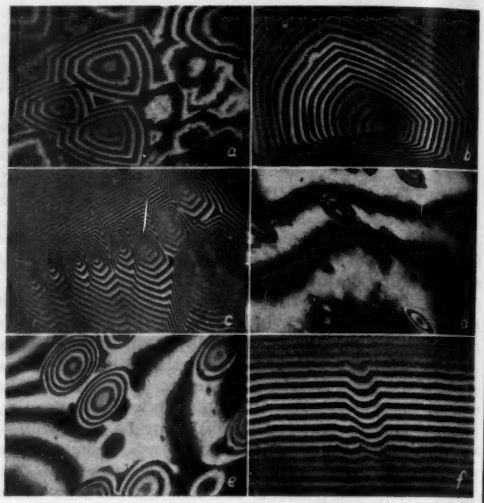

Fig. 1. (*a*) Four-sided etch pits, 20 sec. etching; (*b*) four-sided etch pits, 20 sec. etching, showing isolated hole in curved wall; (*c*) line of overlapping pits, 60 sec. etching; (*d*) boat-shaped etch pits, 10 sec. etching; (*e*) boat-shaped etch pits becoming rounded after 30-sec. etching; (*f*) rounded bottom of boat-shaped etch pit, 10-sec. etching. (× *c.* 40)

It is most noticeable, in these interferograms, how precipitous is the edge of a pit; there appears to be no rounding of the fringes at an edge.

I am indebted to the directors of C. Baker, Ltd., at whose works these photographs were obtained.

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[1] Watts, H., *Nature*, **183**, 314 (1959).

Origin of Libyan Desert Silica-Glass

Since first reading of Libyan Desert glass[1], I have been intrigued concerning its origin. In the course of investigation of impurities in the glassy state, the role of trace germanium in fused silica was studied[2,3]. All fused silica produced artificially from natural quartz contains trace germanium impurity. Two different fused silicas investigated contained 0·9 p.p.m. of germanium[3]. It occurred to me that the trace germanium content of Libyan Desert glass might offer a clue to its origin, as no trace germanium would be expected to volatilize selectively (as germanium(II) oxide) during sudden melting and quenching of a high silica-content glass.

Table 1 gives the results of trace analyses of germanium, using a modified method of Schneider and Sandell[4], in Libyan Desert silica-glass, Libyan Desert sandstone, a Libyan Desert quartzite, two Libyan Desert sands, Aouelloul crater 'impactite' glass, two obsidians, the average for tektites from eleven different localities, including all known sources except Billiton (Darwin glass and americanites excluded), and the range for the non-magnetic portions of six meteoritic stones. It is seen that the Libyan Desert silica-glass contains very nearly the same germanium content as Libyan Desert sands. The average germanium content of the four materials from the Libyan Desert (other than glass) is 0·77 p.p.m. This agrees very closely with the value 0·80 for the Libyan Desert silica-glass and is not too different from the value for impact glass from the

Table 1. SPECTROPHOTOMETRIC ANALYSES OF TRACE GERMANIUM IN SILICATES

Silicate	Source	Ge content (p.p.m.)
Libyan Desert silica-glass	25° 2′–26° 13′ N. 25° 24′–25° 55′ E.	0·80
Libyan Desert sandstone	Gebel Qatrani District, north El Fayum, approx. 29° N. 31° E.	0·52
Libyan Desert quartzite	Gebel Qatrani district, north El Fayum, approx. 29° N. 31° E.	0·95
Libyan Desert sand	Gebel Qatrani District north El Fayum, approx. 29° N. 31° E.	0·77
Libyan Desert sand	From dune 50 km. north of Ghat, Libya, approx. 25° N. 10° E.	0·85
Aouelloul Crater glass	20° 15′ N.	0·64
Impactite	12° 41′ W.	
Obsidian	Lipari, Ægean Sea	1·67
Obsidian	Iceland	1·68
Average for tektites from eleven localities		0·23 ± 0·13
Range of non-magnetic portion of six meteoritic stones		0·09 – 3·02

Aouelloul crater. Obsidian, in general, has about twice the germanium content of the Libyan glass. Tektites, with which Libyan Desert silica-glass is sometimes classified, all have lower germanium contents than the latter. However, stony meteorites bracket the value of 0·80.

In Table 2 an analysis of the Libyan glass is compared with that of Aouelloul glass and sandstone, as well as an Ivory Coast tektite. It is seen that its trace elements match the sandstone more closely than even the impact glass and is quite different from the tektite. According to the results of Friedman[6], the Libyan Desert glass contains an order of magnitude higher water than any tektite and three times as much water as the Aouelloul glass. The latter difference may be correlated with the higher silica content of the Libyan glass.

The dark areas in Libyan Desert silica-glass were found by absorption spectral measurements to be due to streaks of ferrous iron. The inhomogeneity, the presence of crystallites, and the streakiness of the ferrous-containing regions indicate that the glass remained molten for only a short time.

I conclude from the chemical and mineralogical content that Libyan Desert silica-glass is terrestrial in origin, being formed from Libyan Desert materials, most likely by impact of some cosmic body. In this regard the area around 22° 18′ N. and 25° 30′ E., containing explosion craters (as well as volcanic evidence)[9], should be more thoroughly investigated. Ehmann and Kohman[10] indicate a cosmic origin for Libyan Desert silica-glass based on the apparent presence of aluminium-26 and beryllium-10. However, the level of activity of aluminium-26, for which the measurement was the more reliable, is lower than that in chondrites and in australite, and may be due to some other cause than free-space cosmic radiation.

Table 2. ANALYSES

Element (oxide)	Libyan Desert silica-glass*	Aouelloul: Sandstone‡	Aouelloul: Glass‡	Ivory Coast tektite§
SiO_2	98·20	95·30	86·10	76·56
TiO_2	0·23	0·55	0·60	0·60
Al_2O_3	0·70	1·85	5·05	11·54
Fe_2O_3	0·53	0·45	1·45	0·17
FeO	0·24	0·05	1·45	3·99
NiO	0·02	Nil	0·025	—
MnO	—	0·02	0·05	0·08
MgO	0·01	0·40	1·50	3·60
CaO	0·30	0·80	0·90	1·62
Na_2O	0·33	0·20	0·05	1·32
K_2O	0·02	0·10	2·05	0·82
H_2O	0·064†		0·02†	0·0047†

Analysts:
* M. H. Hey (ref. 5).
† Friedman, I. (ref. 6); value under Ivory Coast is average of 17 tektite analyses from various localities.
‡ Anal. Centre Technique d'analyse chimique, Paris (ref. 7).
§ Lacroix, A. (ref. 8).

A detailed report on the germanium contents of tektites and stony meteorites will be published elsewhere.

The germanium analyses were performed by Mr. John Anania of this laboratory. I wish to thank Prof. Truman P. Kohman of the Carnegie Institute of Technology for discussion of the cosmic-ray-induced radioactivities, for the samples of Libyan Desert materials, and for the obsidians investigated.

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Pittsburgh, Pennsylvania.
March 23.

[1] Oakley, K. P., *Nature*, **170**, 447 (1952).
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Diurnal Lapse of Signals from *Sputnik III*

THE radio signals from Satellite 1958 δ 2 have been observed consistently on close transits at Blaxland, near Sydney, in Australia, since shortly after its projection into orbit in May 1958. Until recently no definite failure of signal was observed, although the pulsed modulation code has at times been erratic.

Since early in March, however, there have been definite lapses of modulation. Systematic observation with equipment previously described[1] has established that the modulation is now present only when the satellite is actually in sunlight and ceases soon after it enters the Earth's shadow. This can lead to confusion in casual observations as the transmission may be detectable for only part of a transit. In a particular transit it may actually be received by an indirect propagation path when the satellite is approaching or receding, but not during the closest part of that transit when direct transmission would be expected.

An interesting feature is that, on very close transits, in the absence of the pulse modulation, a weak continuous-wave signal can still be detected with sufficient strength to record the Doppler shift. The rate of Doppler shift definitely establishes the signal as transmission from the satellite. It can thus be very useful for radio tracking in the absence of modulation. This continuous radiation is audible, and visible on the records, as a background when the modulation is present, as can be seen in the diagram in the communication mentioned. The reason for this background radiation would no doubt be apparent if we had a little information on the emitting equipment.

G. H. MUNRO

Radio Research Board Laboratory,
Electrical Engineering Department,
University of Sydney. April 28.

[1] Munro, G. H., and Heisler, L. H., *Nature*, **183**, 809 (1959).

FORTHCOMING EVENTS

*(Meeting marked with an asterisk * is open to the public)*

Tuesday, June 2

BONE AND TOOTH SOCIETY (at the Institute of Orthopædics, 234 Great Portland Street, London, W.1), at 2.55 p.m.—Annual General Meeting, followed by Scientific Papers.

UNIVERSITY OF LONDON (at the Institute of Archæology, Inner Circle, Regent's Park, London, N.W.1), at 6 p.m.—Prof. C. M. Robertson: "Animals in Ancient Greece".* (Fifth of six lectures on "Animals in Archæology". Further lecture on June 9.)

Wednesday, June 3

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Mr. D. Stafford-Clark: "The Contribution of Psychiatry to Modern Medicine".

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. N. Rast and Mr. R. V. Beavon: "The Nomenclature and Diagnostic Characters of Ignimbrites, with reference to Snowdonia"; Dr. D. S. Weedon: "The Gars-bheinn Sill, Isle of Skye".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. C. F. Powell, F.R.S.: "The Origin of Particles of Great Energy in the Cosmic Radiation".

Thursday, June 4

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Prof. D. M. S. Watson, F.R.S.: "The Myotomes of Acanthodians"; Mr. G. R. Coope: "A Late Pleistocene Insect Fauna from Chelford, Cheshire".

ROYAL ANTHROPOLOGICAL INSTITUTE (at 21 Bedford Square, London, W.C.1), at 5.30 p.m.—Mr. W. B. Fagg: "A Field Report on Yoruba Art".

POLAROGRAPHIC SOCIETY (at "The Duke of York", 8 Dering Street, London, W.1), at 7 p.m.—Informal Discussion Meeting on "Some Polarographic Problems Encountered in a Private Consulting Practice" introduced by Mr. F. Kenyon.

CHEMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 7.30 p.m.—Scientific Papers.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned.

ASSISTANT LECTURER (with a science degree, with special qualifications and preferably postgraduate experience in entomology or helminthology) IN ZOOLOGY—The Secretary, West of Scotland Agricultural College, 6 Blythswood Square, Glasgow, C.2 (June 5).

LABORATORY STEWARD (over 21 years of age, with previous laboratory experience, and preferably experience in the preparation of biological materials and in photography) IN THE DEPARTMENT OF BOTANY—The Registrar, University College, Singleton Park, Swansea (June 6).

LECTURER (with research experience in pure mathematics) IN MATHEMATICS—The Secretary and Registrar, University College of North Wales, Bangor, North Wales (June 6).

PRINCIPAL (with high academic qualifications and teaching and organizing experience in a university department, grammar school or technical college) OF CROYDON TECHNICAL COLLEGE—Chief Education Officer, Education Office, Katharine Street, Croydon (June 6).

SENIOR LECTURER (with a degree in chemistry or pharmacy and a postgraduate qualification) IN THE PHARMACY DEPARTMENT, to direct research and to undertake a limited amount of postgraduate teaching—The Registrar, The Technical College, Sunderland, Co. Durham (June 6).

RESEARCH FELLOW (with a Ph.D. degree or equivalent research experience in physical-organic chemistry or biochemistry) IN THE CHEMISTRY DEPARTMENT, for an investigation of the mode of action of germicides—The Professor of Chemistry, The University, Hull (June 8).

RESEARCH FELLOW (with a Ph.D. degree or equivalent research experience) IN THE CHEMISTRY DEPARTMENT, for work on adsorption from the liquid phase by carbon adsorbents, in collaboration with Dr. J. J. Kipling—The Professor of Chemistry, The University, Hull (June 8).

LECTURER (graduate in either physics or engineering) IN THE DEPARTMENT OF PHYSICS, either to develop electronic instruments for application in nuclear physics or to undertake development work on the Department's synchro-cyclotron or other accelerating machines—The Registrar, The University, Liverpool (June 10).

LECTURER (with qualifications in genetics, preferably with emphasis on animal genetics) IN ANIMAL GENETICS—The Secretary and Registrar, University College of North Wales, Bangor, North Wales (June 10).

SENIOR LECTURER IN THE DEPARTMENT OF ANATOMY, to be primarily responsible for the organization and teaching of anatomy in courses for postgraduate students—The Secretary, The University, Edinburgh (June 10).

JUNIOR LECTURER (preferably with medical qualifications) IN PHYSIOLOGY—The Secretary, Medical College of St. Bartholomew's Hospital, West Smithfield, London, E.C.1 (June 12).

LECTURER IN MECHANICAL ENGINEERING—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (June 13).

ASSISTANT LECTURER IN PHILOSOPHY—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (June 15).

LECTURER or an ASSISTANT LECTURER IN THE DEPARTMENT OF PHYSICS—The Deputy Registrar, The University, Birmingham 15 (June 16).

GEOLOGIST (with an honours degree in geology) at the New Zealand Oceanographic Institute of the Department of Scientific and Industrial Research, Wellington, for research in problems of marine geology and particularly those of sedimentation—The High Commissioner for New Zealand, 415 Strand, London, W.C.2, quoting Ref. No. B.11/21/8 and mentioning *Nature* (June 17).

LECTURER IN CHEMISTRY, a LECTURER IN GEOLOGY, a LECTURER (with a good honours degree in electrical engineering or physics, and preferably research experience in solid-state electronics) IN ELECTRICAL ENGINEERING, a LECTURER IN MATHEMATICS, a LECTURER IN PSYCHOLOGY, an ASSISTANT IN PHYSICAL or INORGANIC CHEMISTRY, and an ASSISTANT IN BIOCHEMISTRY at Queen's College, Dundee—Joint Clerk to the University Court, The University, St. Andrews (June 17).

ASSISTANT LECTURERS IN BOTANY (2), CHEMISTRY, and PSYCHOLOGY—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (June 19).

ASSISTANT LECTURER IN PHYSICS—The Registrar, The University, Manchester 13 (June 20).

LECTURER (preferably with a knowledge of the theory and practice of mechanisms and automatic controls) IN THE POSTGRADUATE DEPARTMENT OF APPLIED MECHANICS—The Registrar, The University, Sheffield (June 20).

DEMONSTRATOR (ASSISTANT LECTURER) IN PHYSIOLOGY—The Registrar, The University, Leeds 2 (June 22).

CHAIR OF THEORETICAL PHYSICS in St. Salvator's College—Joint Clerk to the University Court, College Gate, St. Andrews (June 27).

DEMONSTRATOR IN THE DEPARTMENT OF ORGANIC CHEMISTRY—The Registrar, The University, Leeds 2 (June 30).

HEAD OF THE DEPARTMENT OF PURE AND APPLIED PHYSICS—The Registrar, Royal Technical College, Salford 5, Lancs (July 1).

SENIOR LECTURER for postgraduate teaching in the DEPARTMENT OF PATHOLOGY—The Secretary, The University, Edinburgh (July 1).

LECTURER IN PSYCHOLOGY at the University of Tasmania, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, July 6).

SENIOR LECTURER IN ORGANIC CHEMISTRY at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (July 31).

ASSISTANT LECTURER (with an honours degree and interested in research work) IN THE BIOLOGY SECTION of the School of Pharmacy—The Secretary-Treasurer, Royal College of Science and Technology, George Street, Glasgow.

BIOMETRICIAN (graduate in mathematics with an interest in biology) IN THE SECTION OF STATISTICS, for duties involving consultation and research on the design and analysis of experiments—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 59/7.

RESEARCH ASSISTANT (with a degree or diploma in agriculture or allied subjects with some interest in grassland), for work in connexion with grassland, with some lecturing duties—The Principal, Seale-Hayne Agricultural College, Newton Abbot, Devon.

SENIOR GEOLOGIST (preferably with a Ph.D. degree in geology and experienced in subsurface stratigraphy, and an interest in economic geology) IN THE RESEARCH SECTION of the Petroleum and Natural Gas Branch of the Department of Mineral Resources, Regina, Saskatchewan—The Agent-General for Saskatchewan, 19 Chester Mews, London, S.W.1.

SENIOR PHYSICS MASTER—The Rector, Glasgow Academy, Glasgow, W.2.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

British Insects: a Simplified Key to the Orders. By P. Skidmore. Pp. 20. (Manchester: Flatters and Garnett, Ltd., 1958.) *2s. 6d.* [33

Agricultural Research Council: Soil Survey Research Board. Soil Survey of Great Britain—Report No. 10, 1957. Pp. iv+43. (London: H.M. Stationery Office, 1958.) *5s.* net. [33

Field Studies Council. Annual Report, 1957–1958. Pp. 28+4 plates. (London: Field Studies Council, 1959.) [33

Imperial Forestry Institute, University of Oxford. Thirty-fourth Annual Report, 1957–58. Pp. 28. (Oxford Imperial Forestry Institute, The University, 1959.) [33

Research and Development at the Heston Laboratories of Atomic Power Constructions Limited. Pp. 10. (London: Atomic Power Constructions Limited, 1959.) [33

Ministry of Education. The Structure of the Public Library Service in England and Wales: Report of the Committee appointed by the Minister of Education in September 1957. Pp. iv+57. (Cmnd. 660.) (London: H.M. Stationery Office, 1959.) *3s. 6d.* net. [43

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